The Resilience of Calcareous Grasslands to Climate Change

Thesis

The resilience of calcareous grasslands to climate change

Thesis submitted for the degree of Doctor of Philosophy

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Abstract

Calcareous grasslands are of high value for a number of ecosystem services, including biodiversity. Soil depth is generally overlooked as an important factor in ecosystem stability, but was found to mediate the effects of change in other environmental variables. A multiscalar approach was used to investigate the interaction of soil moisture, nitrogen availability and form, and soil depth, using a mesocosm experiment, a field-based experiment, and a landscape survey. The field-based experiment also served as verification for a new long-term climate change platform (RainDrop) at Wytham, Oxon.

Soil moisture

- The open-field experiment showed that soil moisture availability significantly influenced both above- and below-ground productivity (Chapter 2).
- An inverse relationship exists between species richness and mean annual rainfall on calcareous grasslands, due to a relative decline in herbaceous forb and legume species, and a corresponding increase in dominance by graminoid species on wetter sites (Chapter 4).

Nitrogen

- Nitrogen deposition did not significantly affect species richness on surveyed sites (Chapter 4) or at the RainDrop experiment (Chapter 2).
- Plant productivity at local community scale showed no significant response to nitrogen additions, though total mesocosm biomass increased with both NOx and Nred in shallow soils; responses were dependent on species identity (Chapter 3).

Soil depth

- Species richness increased with increasing heterogeneity in soil depth (range, standard deviation) (Chapter 4)
- Total biomass increased in deeper soil mesocosms (Chapter 3).

Mediation effect of soil depth

- Site-level soil depth variability increased species richness responses to temperature and precipitation; species richness was higher in more variable soils, and also increased with increasing temperature at a faster rate than sites with less variable soil depth (Chapter 4).
- Heterogeneity in soil depth was a positive influence on species richness and productivity, indicating that variation in habitat has potential to support species richness under climate change (Chapters 3 and 4).

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"Say not, "I have found the truth," but rather, "I have found a truth." – *Kahlil Gibran*

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Presentation of material

Parts of this thesis have been presented:

Talks

Stone, M.J., Gowing, D.J.G., Stevens, C.J. and Maseyk, K. *Laying the foundations: Construction and first results from a long-term climate change experiment*. Joint ECT-PSE Annual Science Conference, Buxton, Derbys. 21-22 May 2019.

Posters

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Stone, M.J., Gowing, D.J.G., Stevens, C.J. and Maseyk, K. *Interaction of environmental factors on a model calcareous grassland community.* BES Annual Conference, Birmingham. 17-19 December 2019.

Chapter 1 Calcareous grassland

Water is a key resource that determines plant community composition (Silvertown, Araya and Gowing, 2015), as soil moisture regimes determine the productivity of, and competition within, plant communities. Climate change is likely to alter the moisture regime both annually and seasonally, and therefore there is a need to investigate how vegetation types of conservation importance such as calcareous grasslands are likely to respond.

Other environmental factors interact with climate, so it is also important to consider how changes in one system will influence other key systems. Chief among these is the availability of plant nutrients, and in particular nitrogen, which is the major plant nutrient. Nitrogen availability often limits plant growth in terrestrial ecosystems, and is influenced by soil moisture regimes (Araya, Gowing and Dise, 2013). The interaction of water regime with nitrogen supply and availability is, therefore, a significant aspect of investigations into the effect of climate change.

1.1 Calcareous grassland – an overview

Calcareous grassland occurs predominantly on the shallow, well-drained infertile soils that form over limestone. It is a threatened habitat with high biodiversity, which has suffered decline and degradation primarily through land use changes, including reduced grazing levels, total abandonment, and agricultural improvement. Due to its diverse nature, and the large number of rare and specialist species it supports, calcareous grassland is a priority habitat type requiring conservation action under the UK Biodiversity Action Plan (JNCC, 1994); internationally, it is an EU Habitats Directive Annex I habitat.

In the UK, the National Vegetation Classification system (Rodwell *et al.*, 1992) describes 14 types of calcareous grassland (Table 1.1); ten of these (CG1-CG10) are predominantly found in lowland settings (i.e. below the upper limit of cultivation), while the remaining four are higher altitude communities. These habitats provide a wide range of ecosystem services, including animal forage (hay and pasture), soil stability, carbon sequestration and contribute to continued provision of potable water, as well as being important for biodiversity.

Table 1.1 NVC calcareous grassland habitats.

NVC calcareous grassland habitats	
CG1	*Festuca ovina-Carlina vulgaris* grassland
CG2	*Festuca ovina-Avenula pratensis* grassland
CG3	*Bromus erectus* grassland
CG4	*Brachypodium pinnatum* grassland
CG5	*Bromus erectus-Brachypodium pinnatum* grassland
CG6	*Avenula pubescens* grassland
CG7	*Festuca ovina-Hieracium pilosella-Thymus praecox/pulegioides* grassland
CG8	*Sesleria albicans-Scabiosa columbaria* grassland
CG9	*Sesleria albicans-Galium sterneri* grassland
CG10	*Festuca ovina-Agrostis capillaris-Thymus praecox* grassland
CG11	*Festuca ovina-Agrostis capillaris-Alchemilla alpina* grass-heath
CG12	*Festuca ovina-Alchemilla alpina-Silene acaulis* dwarf-herb community
CG13	*Dryas octopetala-Carex flacca* heath
CG14	*Dryas octopetala-Silene acaulis* ledge community

The NVC is a useful tool for describing communities based on species that are commonly found together, though rarely are there distinct boundaries between types, rather a diffusion from one to another as habitat boundaries blur due to species overlap and environmental gradients. It corresponds well with international schemes of habitat classification, such as EUNIS, allowing comparison across political and geographical boundaries. The NVC is not prescriptive but descriptive, and is based on species identification with no overt information about the processes driving those species groupings; other systems based on functional groupings are more useful when trying to understand how communities may change in the face of environmental perturbations.

1.2 Defining diversity of calcareous grasslands

The fundamental measure of diversity is the species count, or species richness, metric; diversity indices take into account factors such as abundance or frequency, and evenness of the distribution of abundance across the species present in a community. Species richness and diversity indices give a measure in terms of number of species and their relative abundance, but tell us nothing about the character of species that are present, or whether prevailing conditions are optimum or even preferable for the species present. Calcareous grassland is a low nutrient ecosystem, composed predominantly of slow-growing species, within which changes in frequency and abundance may be slow and hard to detect through single time-point surveys. Key to understanding why certain species are experiencing expansion or decline is identifying the processes and pressures driving those changes (Adler *et al.*, 2011).

A trait-based approach to plant community diversity allows a better understanding of changes in community composition and dynamics both spatially and temporally, than consideration of a community's taxonomic composition alone (Violle *et al.*, 2007). Traits such as life history, plant group, phenology and physical metrics (height, specific leaf area, seed size) can be used to define functional groups, which can then be used to describe species' fitness in terms of their growth, reproduction and survival (da Silveira Pontes *et al.*, 2015), or to characterise relationships between species and available resources or prevailing conditions. The most widely used index schemes are Grime's C-S-R strategies (Grime, 1977; Grime, Hodgson and Hunt, 1988; Grime, 2006), and the Ellenberg indicator indices (Hill *et al.*, 1999).

1.2.1 Life history

All species seek to optimise the timing of their reproduction and the number of offspring they produce, and there are trade-offs between survival and fecundity (Rathcke and Lacey, 1985; Friedman and Rubin, 2015). Plant species life histories are concerned with their reproductive strategy, i.e. the time required to bring them to reproductive maturity, and the number of times or seasons over which they are able to reproduce. Perennial species have lifespans of over one year and are able to allocate resources to vegetative growth and reproductive organs across repeated cycles. Species may have the ability to reproduce once (monocarpic) or many times (polycarpic) over their lifespan (Grime, Hodgson and Hunt, 1988). Annual species complete their lifecycle in one year; they characteristically produce large numbers of seeds, and have high rates of seedling development, enabling annual species to propagate and disperse rapidly.

Biennial species incorporate traits of both perennials (multiple seasons of vegetative growth) with annuals (monocarpic) , and occur as early successional species generally in open vegetation that is intermittently disturbed (De Jong, Klinkhamer and Metz, 1987). The biennial strategy enables a species to delay reproduction until growth and development are sufficiently progressed; if conditions act to retard development, they are able to extend their life cycle into a second growing season. Many biennials are functional annuals, able to flower on their primary stems if the growing season and conditions are sufficiently long and favourable (Bernice Smith, 1927). Where perennial species have potential for multiple reproductive cycles, annuals and biennials have one reproductive event, and die after flowering.

1.2.2 Grime's C-S-R

Grime's C-S-R classification of plant species (Grime, 1977) proposed three main drivers of plant species' strategies, based on the three main threats to plant existence: competitive exclusion, chronic stress, and repeated severe disturbance (Grime, Hodgson and Hunt, 1988). Each threat occurs under different environmental conditions, e.g. prevailing drought (chronic stress), shifting substrates on sand dunes or scree slopes (repeated severe disturbance). The three primary classes represent plant species that are adapted to these main drivers; competitors (C) are ecologically specialised to cope better with potential competitive exclusion, stress-tolerators (S) with severe stress, and ruderals (R) where the habitat is frequently and severely disturbed. Grime's triaxial representation of plant strategies can be subdivided from the three primary strategies (C, competitors; S, stress-tolerators; R, ruderals) into secondary and tertiary

intermediate levels. Each class is defined by the relative values of the three primary factors, as plotted along the axes (Figure 1.1).

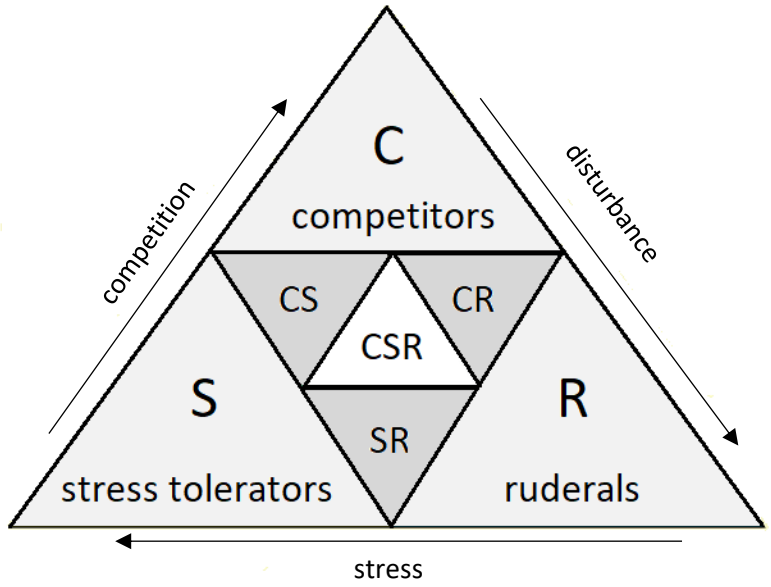


Figure 1.1 Grime's C-S-R triangle - triaxial representation of environmental influences on plant species.

These strategies classify plant species based on how they deal with stress and disturbance. There are three main groups: competitors, which are often tall, large-leafed herbaceous perennials; tough-leaved slow-growing stress-tolerators; and the fast-growing ruderals, which are often annuals and are characteristic of more disturbed habitats.

Typical species of calcareous grasslands are stress-tolerators and competitor/stress-tolerator/ruderal (i.e. adaptive generalist) species (Hunt, Colasanti and Hodgson, 1996; Hancock, 2016). The three main types respond differently to resource depletion – competitive species respond rapidly through morphogenic changes in the distribution of leaves and roots; stress-tolerator responses are slow and small in magnitude; ruderal species rapidly restrict vegetative growth and divert resources into flowering, in order to maximise reproductive potential (Grime, Hodgson and Hunt, 1988)

Grime's C-S-R strategies give us information about the function of species within a community, unconstrained by individual species ID, i.e. they outline the role a species may play in a community. Grime's C-S-R strategies can be used to consider the functional profile of a habitat from its constituent member species, and is often applied as an abundance- or frequency-weighted mean of the C, S and R components of those member species.

1.2.3 Ellenberg environmental indicators

Ellenberg indicator values (EIVs) give information about the physical and chemical setting of a habitat, i.e. a species' ecological niche. Ellenberg N indicates soil nutrient status, with higher values indicating increasing fertility, which can be linked to nitrogen deposition. Ellenberg N values can be used as a proxy for soil fertility, and are loosely correlated with Grime's stress characteristics (2006). In general, low nutrient levels correspond to plants with high stress tolerance. Ellenberg F refers to moisture availability; wetter habitats have higher Ellenberg F values. Ellenberg R is linked to the overall community tolerance for acidity, which is linked to soil pH; low Ellenberg R correspond to low pH, i.e. lower values are more acidic. Ellenberg L gives an indication of light availability; lower values indicate increasing degrees of shade.

Though the use of mean values for EIVs has been criticised (Zelený and Schaffers, 2012) , this has been and continues to be a useful way of gaining information about community-level performance and its response to abiotic factors, provided they are used mindfully (Tölgyesi, Bátori and Erdos, 2014; Schaffers and Sýkora, 2000; Carroll *et al.*, 2018). Originally drawn up for a central-European flora, Ellenberg's original values for species were revised for use in the UK (Hill *et al.*, 1999), to better reflect species' distribution and behaviour in UK habitats. The original "N (nitrogen)" indicator has been replaced by a nutrient or productivity index, as proposed by Hill and Carey (1997) and supported by Schaffers and Sýkora, (2000), who found a stronger correlation between Ellenberg N values and biomass production than with any soil characteristic. Schaffers and Sýkora (2000) also suggested using "calcium values" instead of "reaction" (Ellenberg R), as the pre-exiting metric (wide species tolerance for intermediate levels of pH) meant that mean indicator values were similar for all sites with pH > 4.5, making differentiation difficult for sites with calcareous or neutral soils. EIVs as calculated or estimated by Hill *et al.* (1999) have been used throughout this thesis, and definitions of ecological tolerances can be found in Appendix 1.

Typical species of calcareous grassland communities are stress-tolerator and competitor/stress-tolerator/ruderal species (Hunt, Colasanti and Hodgson, 1996), so a habitat C-S-R signature diverging from this would suggest a compositional change towards a different community, and thus is an indication of less-typical environmental conditions for calcareous grassland. Similarly, an ecological niche can be identified from mean trait values such as Ellenberg indicators (da Silveira Pontes *et al.*, 2015).

1.3 Threats to calcareous grasslands

The calcareous grassland habitat has evolved under restricted conditions of pH, nutrient input and water availability, and variation in these conditions has potential to influence species and functional composition of the plant communities. Up until recent decades, land use changes have posed the major threat to calcareous grasslands, through both agricultural improvement and abandonment (WallisDeVries, Poschlod and Willems, 2002). Abandonment has led to the encroachment by shrubs and subsequent reforestation in areas where traditional grazing has declined of ceased (Poschlod and WallisDeVries, 2002). The addition of organic matter or chemical fertilisers as part of agricultural improvements, along with the deposition of atmospheric nitrogen products, has been shown to be associated with a general degradation of the calcareous grassland habitat, and the selective loss of species adapted to infertile conditions (Stevens *et al.*, 2006; Maskell *et al.*, 2010; Stevens, Duprè, *et al.*, 2011; Payne *et al.*, 2013; Stevens *et al.*, 2016; Soons *et al.*, 2017). Eutrophication due to excess nitrogen availability has been implicated in the decline of characteristically xeromorphic grassland species through competitive exclusion by more mesotrophic species such as *Arrhenatherum elatius, Bromus erectus* and *Brachypodium pinnatum*; these are species better able to exploit this increased nutrient resource (Bobbink, 1991; WallisDeVries, Poschlod and Willems, 2002; Poniatowski *et al.*, 2018).

Overlying the effect of changes in management, and interlinked with nitrogen deposition rates, are those relating to the physical environment, and, in particular, to moisture availability. The calcareous grassland habitat is strongly water-limited; the plant community is typically composed of slow-growing and drought-resistant species that have low nutrient requirements and tolerances. Although there is a strong tendency towards drought-resistance in these communities, it is not clear how they will respond to the combined effects of increased nutrient availability (driven by nitrogen deposition) and greater extremes in water availability that projected emissions and climate models suggest are likely in the near future (NEGTAP, 2001; RoTAP, 2012; Gohar *et al.*, 2018).

Resilience has variously been described as “the ability of a community to maintain its composition and biomass in response to environmental stress” (Grime *et al.*, 2000), “the capacity of a system to persist in the same state in the face of perturbation” (Smith, Diaz and Winder, 2017), and the speed of recovery from perturbation (Tilman and Downing, 1994). Resilience is closely aligned to resistance, which is the ability of an ecosystem’s members to acclimate to or tolerate environmental change. Diversity is considered to confer both resilience

and resistance (Tilman and Downing, 1994; Oelmann *et al.*, 2011) as more diverse ecosystems are more likely to include species that are able to persist in the face of environmental disturbance, and therefore to maintain functional integrity. Higher functional diversity also protects against invasion from species in otherwise missing functional groups (Byun, de Blois and Brisson, 2013). The ability to predict habitat resilience depends on which aspects of the habitat are being investigated (Hirst *et al.*, 2005); it can refer to the rate of ecosystem recovery following disturbance, and also to an ecosystem's ability to reorganise itself. For example, reduced biomass production due to the loss of a dominant forb through an inability to tolerate extended summer drought (lack of resistance) can be compensated for by an increase in compensatory growth and expansion by other member species in subsequent seasons (Hoover, Knapp and Smith, 2014).

1.3.1 Potential influence of projected climate changes

Climate change is irrefutable and is already implicated in range and phenological changes across a wide range of taxa. Climate change models predict continued increases in mean global surface temperatures above those already seen, and increasing perturbation of natural climate cycles. Mean annual temperature in the UK between 2009 and 2018 have been 0.3 °C higher than the 1981-2010 average; the ten warmest years since 1884 have all been since 2002, culminating in the highest summer temperature officially recorded in the UK (38.7 °C on 25 July 2019, Cambridge Botanical Garden) (Met Office, 2019).

Recent models released by IPCC (Gohar *et al.*, 2018; IPCC, 2018; Met Office, 2019) suggest that, for the UK, this warming is likely to see mean annual temperatures rise by up to 3 °C by year 2100, with warmer, wetter winters, and hotter, drier summers. Hot spells (where maximum daily temperatures exceed 30 °C for two or more days) are predicted to increase eight-fold, from an average occurrence rate of one every four years (the rate in 2019), to over four such events every year by 2070. Seasonal phenomena will be less predictable, e.g. summer rainfall projections are in the range of -47% to +2%, with winter precipitation between -1% and +35%. Although the trend in future summer rainfall is to decrease overall, projections from the UKCP Local model (Met Office, 2019) indicate that rainfall events will become increasingly intense.

Interaction of temperature and rainfall with other environmental variables will result in a cascade of effects that have potential to significantly influence plant community composition and function. Resistance to climate change has been defined as "the ability of a community to maintain its composition and biomass in response to environmental stress" (Grime *et al.*, 2000),

and consideration of the composition (species and functional) can help bridge the gap between what we see, i.e. the results of changing climate, and our understanding of the mechanisms and processes leading to those results. Restoration and conservation projects often operate under the assumption that, given the right abiotic ingredients, plant communities have only one destination assemblage; there is increasing evidence that this is not the case, and that it is trait assemblages rather than species assemblages that drive the community composition (Helsen, Hermy and Honnay, 2012).

Water availability is a major limiting factor in calcareous grasslands – typical species are small and slow-growing because endemic drought conditions in the exposed shallow soils, on which this community is found, retard growth and nutrient uptake. Plants take up CO_2 for photosynthesis via open stomata; water vapour is lost through diffusion out of the open stomata at the same time. In drought conditions, a plant will reduce water loss by closing stomata, thus stopping photosynthesis, and ceasing production of new phytomass. Photosynthetic potential is highest during bright sunshine, which is also when the risk of excess water loss is at its greatest; summer drought conditions, then, by forcing repeated and prolonged cessation of photosynthesis to reduce water loss, can have significant impact on productivity. Sustained drought will correspondingly extend the period over which plants are unable to photosynthesise; longer summer droughts in particular will inhibit growth and development, leading to a selection for the most drought-tolerant species, a reduction in competitive species, and a widespread risk of desiccation across all species and functional groups. Risk of wildfire is also enhanced under drought conditions, especially where there is a standing body of dry biomass (upstanding grass flower stalks and other senesced or desiccated plant material.

Grassland productivity has been closely linked to precipitation (Sala *et al.*, 1988; Silvertown *et al.*, 1994; Han *et al.*, 2018), and is a key driver of community structure and function worldwide. Temporal variation in rainfall has been seen to have as much impact on soil moisture as reductions in rainfall amounts (Fay *et al.*, 2003), indicating that seasonality of rainfall is critical for above-ground biomass production (Sala *et al.*, 1988; Clary, 2008). In their long term rainfall manipulation experiment in a water-limited grassland, Fay *et al.* (2003) found that plant community responses to greater variability in soil water content, such as would be caused by longer periods between rainfall events, had the same or greater effect as a 30% reduction in total precipitation

Increased rainfall has potential to alter soil chemistry and structure; soil pH can be lowered when basic ions are leached down the soil profile, causing acidification of the upper horizons. This is ameliorated somewhat by the presence of clay particles, which bond with nutrient cations in the soil solution and prevent them being lost down the soil profile. This process is dependent on the presence of water, and so long-term or extreme changes in soil moisture availability can affect soil pH. This in turn affects plant nutrition, as the different forms of nitrogen are more easily taken up by plants under conditions of different soil pH – ammonium is more readily acquired under low pH, whereas nitrate is predominantly utilised in calcareous soils, which may show deficiency in phosphorus, manganese, iron, boron and zinc.

With increasing mean annual temperatures, species' ranges will shift into higher latitudes and elevations. North-facing slopes may become local refugia, but cold-adapted species with ranges already limited to high latitudes or elevations will effectively run out of space (Freeman *et al.*, 2018). This may lead to extinction for some, and adaptation in others; some Alpine annual species are showing increasing shifts to a biennial strategy with earlier germination as a response to climate change. The impact of meteorological changes on plant communities will depend on when they occur in relation to plant development stages such as spring and summer growing seasons, flowering and fruit set, and seed germination. Range expansion driven by temperature or drought considerations will be restricted to species also able to adapt to changing photoperiodicity (Tomiolo and Ward, 2018), as day length is a common cue for germination and the instigation of plant processes controlling growth and development. Variation in any of these has implications for reproduction, recruitment and immigration (Moser *et al.*, 2011; Maalouf *et al.*, 2012), and, ultimately, for the resulting community composition, where a disjunct develops between two or more critical cues.

1.3.2 The role of nitrogen in shaping calcareous grassland communities

The release of nitrogen and sulphur compounds into the environment through modern industrial and agricultural processes is now recognised as a major threat to ecosystem stability worldwide. Both are important plant nutrients, and their deposition at greatly increased rates is driving changes to the soil-plant system globally, through changes to chemical and biological processes directly involved in plant growth and development, and through soil acidification and the mobilisation of toxic metal ions. Nitrogen is an essential limiting resource for many ecosystems, but it does not follow that an excess can be used, rather, it has been found to cause eutrophication and acidification in affected water and soils (Emmett *et al.*, 2011; Stevens, Duprè, *et al.*, 2011; Phoenix *et al.*, 2012); sulphur dioxide also contributes to acid deposition,

and were found to be the main cause of acidification of Scandinavian lakes as long ago as 1968. Although sulphur emissions have reduced in recent years (RoTAP, 2012), UK progress on reducing nitrogen pollution has been much slower, such that the Committee on Climate Change has warned "that reductions [in nitrogen oxide emissions] have flatlined since 2008" (House of Commons Environmental Audit Committee, 2018). This is despite the wide acknowledgement that enhanced atmospheric nitrogen deposition is now considered "one of the most important components of global change, threatening both the structure and functioning of ecosystems" (Phoenix *et al.*, 2012).

UK emissions figures for 2016 indicated that 99% of nitrogen oxide emissions came from burning fossil fuels; 34% from road transport; 23% from other transport; 12% from other industrial processes. Ammonia emissions were predominantly from agricultural sources, mainly dairy and the application of nitrogen-rich fertilisers. The rates of both emission and deposition are predicted to continue to rise (Smith, Schuster and Dukes, 2016), with higher N availability increasing the potential for damage across greater areas (Bobbink and Hettelingh, 2011; Bobbink *et al.*, 2012).

The relative impact of increased nitrogen availability depends on a number of factors, including the dose rate and cumulative amount of input, and the duration of time over which those inputs continue to be made (Duprè *et al.*, 2010; Stevens, Duprè, *et al.*, 2011; Diekmann *et al.*, 2014); the form of nitrogen (oxidised, reduced) (Stevens *et al.*, 2010; Bobbink *et al.*, 2012); interactions with other environmental and climatic factors, such as rainfall, temperature (Carroll *et al.*, 2003; Lü *et al.*, 2014); and the sensitivity of the species or community under consideration. As sensitivity to perturbation of a particular kind is a core factor in ecosystem response, understanding the need to define degrees of sensitivity led to the development of the critical load concept, as a tool for assessing the risk of different polluting inputs to particular communities and subcommunities (Nilsson and Grennfelt, 1988; Bobbink *et al.*, 2003; Bobbink and Hettelingh, 2011). The critical load is the level below which deposition of a substance has no long-term harmful effect on ecosystem function or structure, so far as current knowledge allows; they are often indicated as ranges of values in order to accommodate variations in physical and ecological factors, and may extend to all the members of a community, or to particular subsets. They are used primarily as indicators of potentially damaging inputs, in order to inform possible mitigation strategies to protect habitats from loss of biodiversity (Wilkins, Aherne and Bleasdale, 2016)

The impacts of nitrogen accumulation, particularly in habitats that have evolved in low nitrogen conditions, include community responses such as an increase in more competitive nitrophilous species – particularly grasses - and an associated decline in diversity, and environmental effects such as increased mineralisation, nitrogen-leaching and surface soil acidification. Ecosystem responses have been observed in surveys along gradients of nitrogen deposition (Hector *et al.*, 1999; Stevens *et al.*, 2004; Duprè *et al.*, 2010; Stevens, Manning, *et al.*, 2011). Such studies have widely found an increasing rate of nitrogen deposition to be associated with declines in biodiversity as nitrophilous species are advantaged and able to expand their range and competitively exclude other species. Physiological responses to increased nitrogen lead to an increased potential for damage from secondary stresses such as increased herbivory (as, for example, nitrogen-rich foliage has greater food value); and an increased susceptibility to drought or frost damage (Sheppard *et al.*, 2008). Changes in species composition and ecosystem function have been found to occur at low doses of N deposition (e.g. 5-10 kg N ha^{-1} yr^{-1}) that are below the lower limit of assigned critical loads (Emmett *et al.*, 2011), indicating that the use of critical loading can only be indicative of a habitat's sensitivity, and that nitrogen-driven changes can occur at chronic low levels of deposition, over time (Phoenix *et al.*, 2012).

Experimental additions onto established grassland communities have provided evidence of both the long and short-term effects of increased nitrogen deposition, and shown that even low doses, if continued over a long period, can significantly affect community composition and environmental conditions. Field manipulation experiments at Wardlow Hay Cop (Morecroft, Sellers and Lee, 1994; Carroll *et al.*, 2003) have given insight into the effect of multi-level doses of nitrogen on calcareous grassland. As with other field experiments, Wardlow Hay Cop included very high doses (up to 140 kg N ha^{-1} yr^{-1}), well above atmospheric deposition rates for the UK but comparable to agricultural application rates. The long-running nature of the experiment has allowed the comparison of short and long-term responses, and also given some indication of recovery responses once treatments were ceased. In the short term, there were no clear changes in vegetation structure or growth, though nitrogen mineralisation rates were found to have increased after two and four years of nitrogen addition treatment (Morecroft, Sellers and Lee, 1994; Carroll *et al.*, 2003). After six years, species richness was greater by 29 species on the untreated control plots compared with those that had received the lowest nitrogen addition (35 kg N ha^{-1} yr^{-1}); nitrogen mineralisation rates increased in step with increasing nitrogen addition rates, but not significantly with the lowest addition treatment, and high nitrification rates were associated with significant acidification of the plots receiving the highest dose (140 kg N $h1^{-1}$ yr^{-1}) (Carroll *et al.*, 2003).

1.4 The importance of long-term experiments and databases

As outlined above, calcareous grasslands are predominantly composed of slow-growing perennial species, so changes to the community composition may take decades to become apparent. Changes in species' range and abundance take place over a backdrop of natural cycles of surge and decline, so long-term studies are needed to allow general trends rather than short-term between-year variations to be identified. That long-term datasets are needed to investigate ecosystem functioning under changing conditions is widely accepted (Franklin, 1989; Tilman, 1989; Inouye and Tilman, 1995; Rees *et al.*, 2001; Yahdjian and Sala, 2002; Weisser *et al.*, 2017), though the definition of "long-term" depends on the time-scale at which critical processes operate, and the time taken for responses to become measurable in the studied habitat (Franklin, 1989; Lindenmayer et al., 2012; Rull, 2014).

Long-term studies are particularly important on low productivity habitats such as calcareous grasslands, as these may evidence a slower rate of response than more productive systems, and short-term studies may not detect longer interval changes (Inouye and Tilman, 1995; Grime *et al.*, 2008) or pick up interannual variation in how plant communities respond to changes (Kardol *et al.*, 2010). Interspecific interactions could be related to intrinsic temporal shifts in slow processes such as succession (Tilman, 1987; Sternberg *et al.*, 1999; Hirst *et al.*, 2005; Helsen, Hermy and Honnay, 2012; Harrison, Gornish and Copeland, 2015), or time-lag (Dunnett *et al.*, 1998) or incremental responses (Duprè *et al.*, 2010; JNCC, 2011; Stevens, Payne, *et al.*, 2016), which might be missed by shorter-term or single-survey studies. Long term studies are also essential for capturing rare or episodic phenomena, such as 100-year drought or extreme precipitation events, and for identifying changes to climate patterns (Franklin, 1989).

"The main benefit of long-term studies is that they allow researchers to address problems that no one has yet imagined. If we are to have any hope of conserving species, we need to understand them, and we need to understand the way they are affected by climate change." (Birkhead, 2014).

1.5 Thesis structure

This thesis aims to investigate the roles of soil moisture and nitrogen availability on community composition and productivity in calcareous grasslands, in order to assess the potential risks posed by climate change. Chapter 2 outlines a new long-term experimental platform and

presents verification of its efficacy and potential to contribute usefully to the ongoing and increasingly important research being carried out into the mechanisms and processes at work in the calcareous community under changing climate and increasing atmospheric nitrogen deposition. Chapter 3 presents results from a mesocosm experiment that investigated above- and below-ground responses of a model grassland community to reduced soil resource availability controlled through soil depth, and the interaction with nitrogen deposition in both oxidised and reduced forms. Chapter 4 associates field survey data of community composition on eleven calcareous grasslands in the UK with prevailing or recent climatic conditions, and attempts to assess the role of soil depth in community responses. Chapter 5 presents a brief summation of the major findings of the three strands of research.

Chapter 2 Verification of a new long-term climate change facility, and vegetation response to experimental manipulation of rainfall and nitrogen additions

This chapter presents verification of RainDrop, a new long-term climate change platform at Wytham, UK, and results of a two-year experiment into vegetation changes in response to the imposed variation in rainfall that formed the inaugural study for the site. The research included assessment of changes in species abundance and above-ground biomass production as the primary metrics.

Biomass data highlighted the importance of seasonality of rainfall to productivity, which confirmed the potential for climate change to disturb these grassland communities through variation in timing of rainfall events, and their magnitude. A change in management from being sheep-grazed to biannual mowing may have influenced some of the changes observed in species' abundance - legumes in particular showed a broad expansion across the whole site, and grass cover declined, mostly due to a contraction in *Trisetum flavescens* after 2016. Detection rates for a nitrogen response were low, suggesting that the influence of soil moisture far outweighed the effect of increased nitrogen / nutrients.

2.1 Context

Recent changes in climate have had widespread impacts on human and natural systems, with observed changes in the frequency and distribution of extreme events since the 1950s. These changes have affected terrestrial and aquatic biomes, and have included a global warming of mean air and ocean temperatures, and an increase in the number of heavy precipitation events across a number of regions (IPCC, 2014). Climate change models for the UK predict overall warming, with median increases in daily temperature of 1 °C to 2 °C across the country with global mean warming of 2 °C, and between 3 °C and 5 °C with 4 °C of global warming (Gohar *et al.*, 2018). Associated changes in precipitation for these levels of global warming indicate wetter winters across the whole country (median increases of up to 20%), and a decrease in summer precipitation of between 20% to 30% that would be stronger in the south (Gohar *et al.*, 2018).

It's well known that biodiversity and other ecosystem properties vary in response to water availability (Anderson, Ritchie and McNaughton, 2007; Clary, 2008; Sardans *et al.*, 2008; Kardol *et al.*, 2010; Harrison, Gornish and Copeland, 2015; Stevens, Ceulemans, *et al.*, 2016; Luo *et al.*, 2017), and the predicted shifts in rainfall patterns are expected to impact the phenology, range, productivity and diversity of plant communities (Duckworth, Bunce and Malloch, 2000; Moser *et al.*, 2011). Such climate change-induced shifts are already being recorded across many biomes at a global scale (Sala *et al.*, 1988; Titlyanova *et al.*, 1999; Bobbink *et al.*, 2012; Gallego-Sala and Colin Prentice, 2013), though whether such shifts are resistible or reversible remains unknown. As the stresses from climate change continue, ecosystems are pushed closer to critical thresholds, becoming more unstable and more extreme in their response to climatic perturbations (Huete, 2016). For every habitat, some critical thresholds will be approached sooner than others, depending on individual major limiting factors in operation, and in order to understand potential direction and magnitude of ecosystem changes, it is important to investigate those primary limiting factors and how they may drive changes in ecosystem function.

Grasslands are sensitive to variations in both the amount and frequency of rainfall (Sala *et al.*, 1988; Eziz *et al.*, 2017). This sensitivity manifests through a number of ecosystem functions, including biomass, species presence and abundance, and diversity. Grassland above-ground biomass has been shown to increase with increased rainfall (Sala *et al.*, 1988; Silvertown *et al.*, 1994; Han *et al.*, 2018), and temporal variation in rainfall has been seen to have as much impact

on soil moisture as reductions in rainfall amounts (Fay *et al.*, 2003), indicating that seasonality of rainfall is critical for above-ground biomass production (Sala *et al.*, 1988; Clary, 2008).

Community composition and structure change as a result of species' different responses to variation in resources, whereby some species are advantaged and others are disadvantaged by those changing conditions (Sternberg *et al.*, 1999). This has implications not only for existing community members, but also for invasion potential by locally opportunistic species, and range-expanding incoming species, as changes in resource availability opens up opportunities for migrating species (Grime, 1977; Thompson *et al.*, 2001; Moser *et al.*, 2011). In this way, local diversity is impacted, which has knock-on effects for ecosystem functions and stability (Chapin *et al.*, 2000; Díaz and Cabido, 2001; Smith, Diaz and Winder, 2017; Weisser *et al.*, 2017).

The influence of diversity on productivity and ecosystem stability has long been recognised (Tilman and Downing, 1994; Tilman, 1999; Thompson *et al.*, 2001; Diacon-Bolli *et al.*, 2012) and loss of diversity has been found to lead to a reduction in productivity in many instances (Silvertown *et al.*, 1994; Tilman and Downing, 1994; Naeem *et al.*, 1996; Tilman *et al.*, 1997; Hector *et al.*, 1999), though the converse cannot be assumed, i.e. an increase in productivity does not necessarily lead to an increase in diversity (Naeem *et al.*, 1994, 1996). Though individual species' responses to changing environmental variables has been well studied for a number of species (e.g. Volk, Niklaus and Körner, 2000; Fay *et al.*, 2002, 2003; Gianoli, 2004; Heschel *et al.*, 2004; Davison *et al.*, 2010; Hui *et al.*, 2018), the possible magnitude and direction of plant community responses to climate change is poorly understood (Kardol *et al.*, 2010; Pratt and Mooney, 2013).

At a community level, observed responses may not be easily interpreted; combined effects of interacting variables impact on an individual plant or species via some physiological traits, while ongoing competitive interactions with other community member species generate pressure from other directions (Tilman, 1996). Altered rainfall regimes have been shown to result in shifts in plant community composition, but there is increasing evidence that the direction of this shift is not fixed, and that it is trait or function assemblages, rather than species assemblages, that drive community composition and provide ecosystem stability (Helsen, Hermy and Honnay, 2012; Van Looy, Lejeune and Verbeke, 2016; Roscher *et al.*, 2019). In order to investigate this aspect of plant communities, species present can be grouped according to particular shared traits such as plant guild or group, e.g. graminoids, legumes, herbs (Tilman, 1987; Tilman *et al.*, 1997; Hector *et al.*, 1999), using Grime's C-S-R classes (Tilman, 1996; Hodgson *et al.*, 1999; Pierce *et al.*, 2013, 2017; Morecroft *et al.*, 2016), or Ellenberg indicator values for environmental

factors (Hill and Carey, 1997; Hill *et al.*, 1999; Bartelheimer and Poschlod, 2016; Stevens, Ceulemans, *et al.*, 2016; Stevens, Payne, *et al.*, 2016; Carroll *et al.*, 2018). Grouping species like this provides different ways of looking at the data; for example, *Dactylis glomerata* is a highly competitive species (C/CSR in Grime's C-S-R classification), whereas *Brachypodium pinnatum* is more stress-tolerant (SC in Grime's classification) (Hill *et al.*, 1999), so they will respond differently to variations in environmental factors, though they are both in the same plant group (grasses). This approach can help to identify the drivers behind range shifts in seen in some species, e.g. the expansion of *Bromus erectus* and *Brachypodium pinnatum* into, and increasing dominance of, calcareous grasslands (Moser *et al.*, 2011).

The changes in temperature and rainfall patterns predicted for the UK under climate change models (IPCC, 2014) will interact with other environmental variables such as levels of atmospheric pollutants. Notable among these is nitrogen, as many terrestrial habitats are nitrogen-limited, and vegetation likely to respond to changing levels of deposition. There is already clear evidence that nitrogen enrichment reduces species richness and increases graminoid cover in many habitats (Duprè *et al.*, 2010; Maskell *et al.*, 2010; UKREATE, 2010; Field *et al.*, 2014; Soons *et al.*, 2017). Many of these studies consider changes in species richness to be a result of increased acidification of the soil, and a loss of lower-pH-tolerant species from the communities. Calcareous soils are generally considered to be well buffered against such severe acidification, and that the eutrophying effect of nitrogen enrichment may be the bigger driver of community change in this habitat. In the long term, species richness may not be significantly altered, and it is through changes in community composition that a nitrogen-enrichment response is measured and species-specific responses come into play (Newton *et al.*, 2012; Diekmann *et al.*, 2014; Stevens, Ceulemans, *et al.*, 2016).

Untangling the effect of nitrogen enrichment from that of other climate variables in field (observational) survey data is not straightforward, as there are different forms of nitrogen (oxidised, reduced; wet, dry) which have variable temporal and geographic distributions. Nitrogen deposition may also be correlated with other climate variables such as mean annual precipitation, mean winter or summer temperature, e.g. Maskell *et al.* (2010). To investigate whether such interactions are significant drivers of change in calcareous grasslands requires experiments with appropriate levels of randomisation, replication and control, which are able to determine causal links between a manipulated variable and a measured response (Tilman, 1989), while considering geographic and ambient climatic variables, past land use history and management (Bullock *et al.*, 1994; Silvertown *et al.*, 1994, 2006).

As has already been stated, there is an acknowledged link between precipitation and productivity on UK calcareous grasslands, whereby reduced precipitation results in reduced productivity. The question of how predicted changes to climate patterns may impact these grasslands prompted a collaboration between the Ecology Continuity Trust, the Open University, Oxford University and the Patsy Wood Trust to develop a new long-term climate change experiment (RainDrop) on existing limestone grassland at Wytham, Oxfordshire. The intention was to establish a resource with an operational life of over 20 years, using rainfall manipulation experiments to simulate some of the modelled predictions - specifically, increased summer drought, and increased intensity of summer rainfall events. Although total precipitation exclusion has been used at other research locations, e.g. Buxton Climate Change Research Laboratory, Derbyshire (e.g. Grime *et al.*, 2000, 2008; Ravenscroft, Fridley and Grime, 2014), this was not to be implemented at the new facility, as the main climate change effect indicated by the IPCC (IPCC, 2014) regarding precipitation was more a modification of, rather than a cessation or radical remapping of, existing climate patterns.

To explore the predicted climate variations of increased summer drought, and increased intensity of summer rainfall events, a novel rainshelter design was implemented. This was designed to reduce received precipitation by c. 50%, and was based on Yahdjian and Sala's (2002) partial exclusion shelter; a coupled redistribution system simultaneously acted to redistribute the intercepted rainfall, intensifying the rainfall event, and thus simulating the two main precipitation-based elements of climate change predicted for the UK.

Species' persistence under the rainfall manipulations will depend on their ability to tolerate or adapt to the changing environmental conditions. When some species are benefitted by climatic variation, other species may be disadvantaged, either through their own physiological requirements or through increased inter-species competition. Although overall community biomass may remain constant due to species expanding their abundance or switching in to replace outgoing or diminishing species, such alterations to community composition are expected to result in changes in species richness and diversity (Tilman, 1996). Diversity was expected to show a negative relationship with moisture availability, as the increase in a major limiting resource for the calcareous grassland habitat (i.e. moisture) was expected to increase competition (H2.1). Variation in diversity may also be reflected in variation in functional diversity, and should be considered in terms of relative abundance of plant groups or other functional groups. Plots receiving enhanced rainfall were expected to show an increase in dominance of tall, competitive grasses, with an accompanying decrease in herbaceous species (Rodwell *et al.* 2007) (H2.2). It was also expected that there would be a shift towards more

competitive species (in Grime's C-S-R scheme) under the enhanced rainfall treatment (H2.3). As the rainfall manipulations theoretically adjusted received rainfall by the same proportion (i.e. +/- c. 50%), it was hypothesised that resulting reductions in biomass under the drought treatments would be proportionally similar to increased productivity under the enhanced precipitation treatment (H2.4).

In order to investigate the interaction of climate change with another major anthropogenic environmental perturbation, nitrogen addition treatments were nested within the rainfall treatments. Adequate levels of soil water are necessary for the uptake and utilization of nitrogen by plants, and as C3 forbs are generally considered to need more soil nitrogen than C4 grasses (Silvertown *et al.*, 1994), it was felt that the interaction of soil moisture and available nitrogen would influence grass:forb ratios. Where the supply of soil moisture is sufficient, nitrogen addition should promote plant growth, resulting in increased biomass compared to plots that do not receive nitrogen. Where soil moisture is not sufficient, the addition of nitrogen should have no or little effect on plant biomass, as plants are unable to make use of it (H2.5). Nutrient addition manipulations were also expected to impact community structure as species and functional groups differ in their response; the grass :forb ratio was used to investigate the interactive effect of variations in precipitation and nitrogen deposition on community composition, though it is not clear whether the combination of simulated climate change and nutrient addition will be additive or complementary, .

Hypotheses tested specific to the plant community were:

H2.1 Diversity will show a negative correlation with received rainfall amount, i.e. will be higher under the drought treatment.

H2.2 Grass to forb abundance ratio will increase under enhanced rainfall treatment compared to the drought treatment.

H2.3 More competitive species will increase in abundance under enhanced rainfall compared to the drought treatment.

H2.4 Reduction in above-ground biomass under the drought treatment will be proportionally similar to an increase in above-ground biomass under the enhanced rainfall treatment.

H2.5 Plot biomass will increase in response to nitrogen addition, where moisture availability is sufficient. Where soil moisture is insufficient for nitrogen-uptake, nitrogen addition will have no effect on plot biomass.

In summary, there was a dual focus to this study:

a) the coupled rainshelter/redistribution system was assessed for suitability as a tool to investigate climate change impacts on calcareous grassland;

b) the plant community responses to the imposed treatments were investigated.

2.2 Study site and experiment design

2.2.1 Location

The study area is situated approximately 5 km northwest of Oxford, UK (NGR SP 4617 0826; lat. 51.7711, long. -1.3321) (Figure 2.1). It is within a small area of early-reversion lowland calcareous grassland known as Upper Seeds, which is part of the Wytham estate. The site lies at the top of a small hill, c. 160 m above mean sea level, and has a south-easterly aspect.

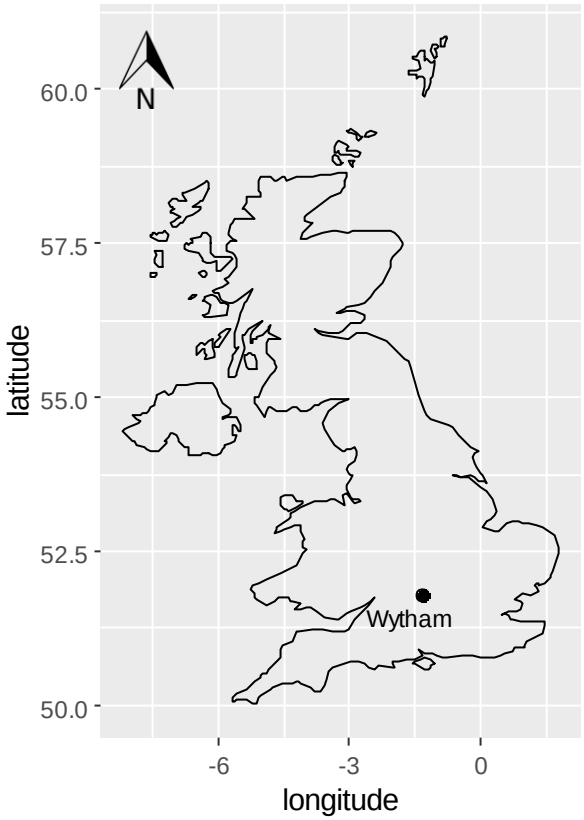


Figure 2.1 Location of study site at Wytham, UK.

Upper Seeds is topographically heterogenous; a plateau lays around the south and western sides, from which the site slopes down towards woodlands to the east, and towards a small hollow in the centre of the site. This hollow is oriented east-west and is possibly associated with water drainage. The south side of the hollow rises most steeply to a level area on which is located an Environment Change Network (ECN) monitoring station.

2.2.2 Geology

Wytham Hill is capped by Corallian limestone laid down in the Oxfordian stage of the Jurassic (c. 160 Ma). This is a fossiliferous sandy limestone that gives rise to clay-rich basic soils.

2.2.3 Soil

The soil at Upper Seeds is part of the Elmton 1 series (soil type 343a, Soil Classification system for England and Wales; (Cranfield University, 2018)), characterised as a shallow, well-drained and brashy calcareous soil overlying soft limestone (Cranfield University, 2004). Post holes excavated on site exposed the general soil profile; this comprised a loam-rich root layer of 2-5 cm, overlying a single layer of sandy clay loam with a maximum observed depth on site of 27 cm. Soil composition is approximately 45% sand, 30% silt, 25% clay.

Soil samples and cores (50 x 80 mm cylindrical cutting tube) were taken from the topsoil horizon below the root layer, to investigate pH, phosphate and moisture retention (see below).

2.2.3.1 Soil pH and phosphate

Soil pH and phosphate were tested before any works commenced on site (Table 2.1), using standard laboratory methods (Olsen *et al.*, 1954). Soil pH was found to be above 7 in all samples taken from Upper Seeds. This places it within the calcareous soil categorisation (pH >7.0), and reflects the compositional dominance of coral sand derived from the underlying limestone.

Phosphate levels are low (i.e. at the lower range of Soil Index 1 (10-15 mg l^{-1} P) (DEFRA, 2010)), indicating an unproductive soil.

Table 2.1 Baseline soil pH and phosphate values. SD = standard deviation of the metric mean. Olsen P is the appropriate test as pH is >7.2.

	Soil pH	**Soil phosphate Olsen-P mg/kg PO_4-P**	**Soil Index Olsen P**
Minimum	7.73	9.58	1
Maximum	7.83	12.04	1
Mean	7.78 (n=15)	11.23 (n=6)	1
SD	0.03	0.86	-

2.2.4 Climate and hydrology

Mean annual rainfall on site (2007-2018) was 745.9 mm; total annual rainfall over the three years of the study were below this 12-year average (Figure 2.2).

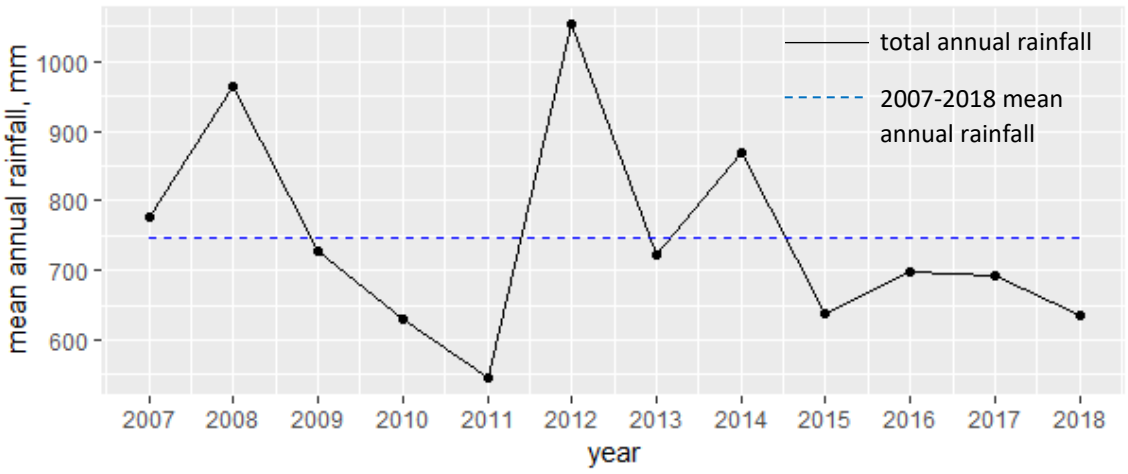


Figure 2.2 Mean annual rainfall: annual total rainfall and 12-year average. Data from ECN AWS T08 2007-2015 (Rennie *et al.*, 2017) *and uncleaned data 2016-2018.*

UK Meteorological Office 30-year averages (1981-2010) show long-term seasonality, with Autumn precipitation being, on average, the highest (average 153.4 mm over the season), with Spring and Summer being both the driest seasons (153.8 and 153.4 mm respectively) (UK climate averages for Oxford, https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages/gcpn7mp10). More recently, seasonality of precipitation has varied over the last 12 years, the highest proportion of annual rainfall fell in the summer months between 2007 and 2012, with the winters being the driest season.

From 2013, this pattern was disrupted by summer drought and an apparent lengthening of the seasonal aspect of rainfall, with wet and dry conditions shifting and extending into following quartiles. 2017 saw a return to summer seeing the highest proportion of precipitation, though the following year (2018) again witnessed severe summer drought (Table 2.2); more precipitation fell in December 2018 than fell over the three summer months combined.

Table 2.2 Total annual and seasonal precipitation for Wytham AWS T08, 2007-2018 (data sources as Figure 2.2). Seasons follow standard UK Met Office definitions: Spring = March-May, Summer = June-August, Autumn = September-November, Winter = December-following February. [a] Winter 2018 precipitation data not known at time of writing, so no annual precipitation total possible. December 2018 precipitation given as (80.5). Seasons with highest proportion of precipitation are coloured blue, seasons with lowers precipitation are sandy coloured.

year	total annual precipitation (mm)	seasonal precipitation (mm)			
		Spring	Summer	Autumn	Winter
2007	760.2	166.4	248.2	155.8	189.8
2008	937.0	257.8	309.0	231.6	138.6
2009	745.6	131.8	229.8	178.6	205.4
2010	638.0	117.6	207.8	160.4	152.2
2011	490.0	72.6	182.0	96.0	139.4
2012	1122.9	224.2	354.6	289.0	255.1
2013	840.9	172.3	88.0	215.5	365.1
2014	720.6	205.6	177.2	177.8	160.0
2015	669.5	120.7	156.4	181.6	210.8
2016	657.3	212.9	142.1	167.1	135.2
2017	677.53	142.73	191.9	166.2	176.7
2018	[a]	247.2	71.6	147.0	[a] (80.5)

Water-holding capacity of the soil was assessed using soil cores taken from the site in March 2016, as part of the general characterisation of the site. The cores were subjected to increasing gravimetric tension on a sand table, from a saturated state to a maximum of -10kPa, and cumulative water loss plotted against the hydraulic tension (whereby 10 cm ≈ 1 kPa) (Figure 2.3).

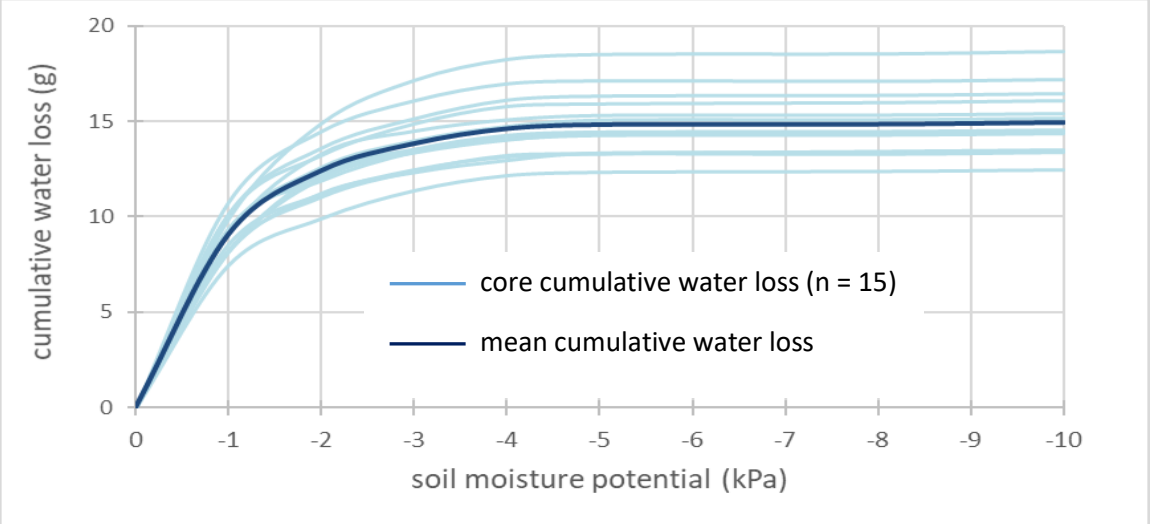


Figure 2.3 Soil moisture release curve for soil cores taken from RainDrop site, March 2016.

2.2.5 Habitat

Upper Seeds is an early reversion calcareous grassland, lying within woodland on the Wytham estate. It has had a varied past, including arable, and more recently, as sheep pasture. Mixed deciduous woodland lies immediately adjacent to the east, and within 100 m to the north. The flora of the Wytham estate has been documented extensively and periodically since 1880 (Gibson, 1986), and species lists for Upper Seeds itself are known not only from previous experiments on the nearby TIGER plots (Grime *et al.*, 2000) but also subsequently, as ongoing monitoring of the estate's ecology. In 2016, the vegetation was dominated by grasses *Trisetum flavescens*, *Arrhenatherum vulgare* and *Brachypodium pinnatum*, wild basil *Clinopodium vulgare* and the legumes *Lotus corniculatus* and *Trifolium repens*, which between them accounted for over 60% of aerial cover in survey quadrats.

Early observations confirmed a number of grassland species on site, such as Black Medick (*Medicago lupulina*), Cowslip (*Primula veris*), Hairy Violet (*Viola hirta*) , Common Centaury (*Centaurium erythraea*) and Field Scabious (*Knautia arvensis*). The site's species list also included plants associated with the previous management history, e.g. Small-flowered Buttercup (*Ranunculus parviflorus*), an annual of neutral grasslands that shows long persistence in seed banks, and some reflecting the prior scrub encroachment (since cleared), e.g. herbaceous perennials Germander Speedwell (*Veronica chamaedrys*) and Ground-Ivy (*Glechoma hederacea*), and the tussock-forming perennial grass False Brome ((*Brachypodium sylvaticum*). Remnant woodland species Cuckoo-pint (*Arum maculatum*) and Bluebell (*Hyacinthoides non-scripta*) have continued to persist in some areas.

Comparison of vegetation data from the baseline survey (June 2016) carried out using the MAVIS software (Smart *et al.*, 2016) indicated that the Upper Seeds community at that time was closest to MG1 (mesotrophic grassland dominated by *Arrhenatherum elatius*), though there was variation within the community suggesting that it was atypical in places, and could perhaps be better described as an MG1-CG4 mosaic (CG4 is the NVC classification for calcareous grassland dominated by *Brachypodium pinnatum*, associated with low level grazing, which was dominant on the north-eastern part of the site). The classification as a MG1-CG4 matrix reflects both the influence of the mixed history of management and nutrient inputs to the site, and, perhaps, its tendency to revert to a CG community due to abiotic environmental factors such as underlying geological substrate and shallow soil depth. Although this classification means that Upper Seeds is not fully representative of wider calcareous grasslands, the high degree of species and

trait diversity present within the vegetation on site echoes the diversity found in more typical CG communities, and so has value for this study in its potential for responsiveness to varying treatment effects.

2.2.6 Historical and present management

In common with many grassland areas across the country, Upper Seeds was ploughed during WW2 and put down to arable; after the war, it was put back to pasture. From 1960 until 1982, it was again under arable crops; management ceased between 1982 and 1984, from when it was wild-grazed only (deer, rabbits, etc.). A conservation grassland management plan was agreed with Natural England in 2002, and sheep were introduced onto the site in November 2006. Spring/Summer and Autumn grazing, along with the occasional use of a forage harvester served to remove and prevent the encroachment of scrub, especially hawthorn, and encourage the calcareous grassland community to establish. Sheep were removed from Upper Seeds in early 2016 to allow for the construction of the experimental platform; thereafter, it has been mown twice annually (midsummer and autumn/winter), and all arisings removed off-site.

2.2.7 Experimental platform (RainDrop): design

To help address the need for long-term climate change experiments, the RainDrop platform was established on Upper Seeds in 2016, as a collaboration between the Ecological Continuity Trust, Open University, Oxford University and the Patsy Wood Trust, and with support from the British Ecological Society. This was intended as a long-term resource investigating how grassland communities may respond to climate change, using rainfall manipulation experiments via a combination of rainshelters and supplemented rainfall to simulate modelled predictions. It is also part of the International Drought Experiment, which follows a standardised protocol drawn up by Drought Net to allow cross-project and cross-boundary comparisons (DroughtNet, 2017).

As well as the two core treatments required by Drought Net – drought and ambient control – two further treatments have been adopted, being enhanced rainfall, and a procedural control. These four treatments were allocated to one plot each, within five replicate blocks on the least topographically diverse areas of the site (blue blocks, Figure 2.4). Three further blocks were outlined for possible future use, but are not currently active (red blocks, Figure 2.4).

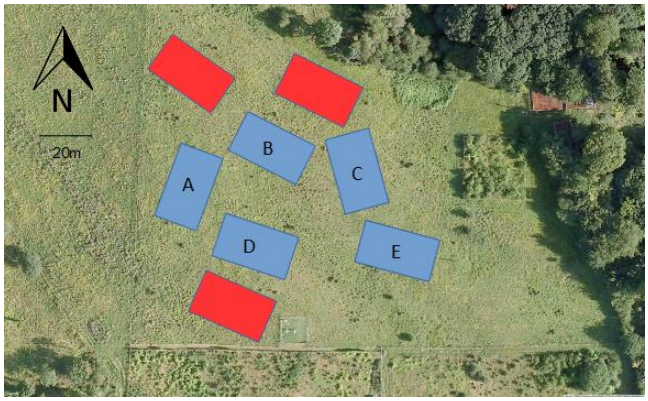


Figure 2.4 Selected blocks (blue, labelled A to E); unused blocks are coloured red and remain unlabelled.

Each block comprises eight 5 m x 5 m experimental plots, with a buffer strip of minimum 1.5 m between them. Within each block, the four principal treatments were randomly allocated, with the single exception that irrigated plots were forced to occupy a plot adjacent to that block's drought plot, for practical purposes. The plots are oriented north-south within the blocks, in order to reduce confounding factors of shading and prevailing weather patterns across the site.

2.2.7.1 Rainfall manipulation treatments

The experiment comprises four principal experimental treatments: two rainfall manipulation treatments – summer drought and increased summer rainfall – and two control treatments - ambient, and procedural (Figure 2.5).

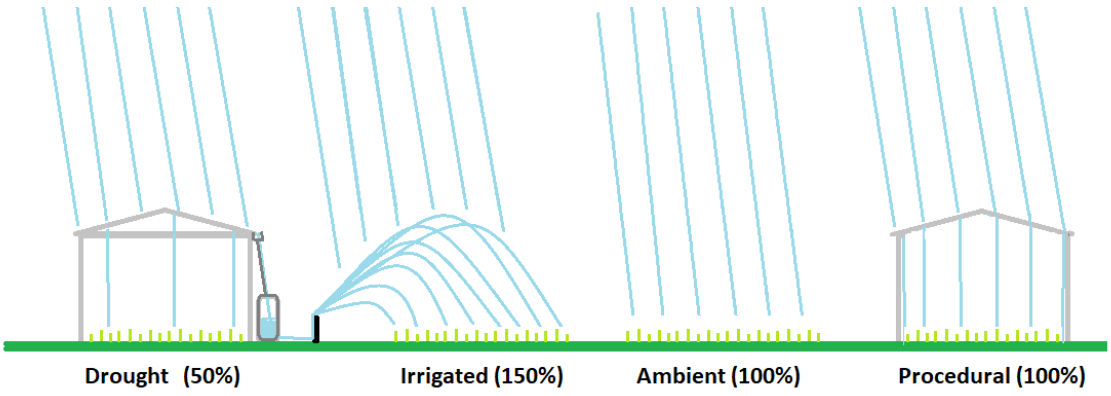


Figure 2.5 Schematic of rainfall manipulation treatments.

Summer drought was simulated by a 50% reduction in rainfall over the experimental plots. This was achieved through the use of a 5 m x 5 m rainshelter; upturned V-shaped gutters (vvvv) on the roof panels are designed to intercept 50% of ambient rainfall, and route it to a storage vessel. This intercepted rainwater was then used to supplement ambient rainfall on an adjacent

plot, simulating greater rainfall intensity while maintaining the natural frequency and duration of precipitation events (cf. Pratt and Mooney 2013) (Figure 2.6).

Procedural control plots comprised a 5 m x 5 m rainshelter with downturned V-shaped gutters (^^^^), designed to allow all ambient rain to pass through unimpeded. The procedural control was intended as a means of assessing the role of the rainshelter structure to changes in untargeted environmental variables, such as temperature and photosynthetically-active radiation (PAR) in the ecosystem response. Ambient control plots were open plots receiving 100% of ambient precipitation.

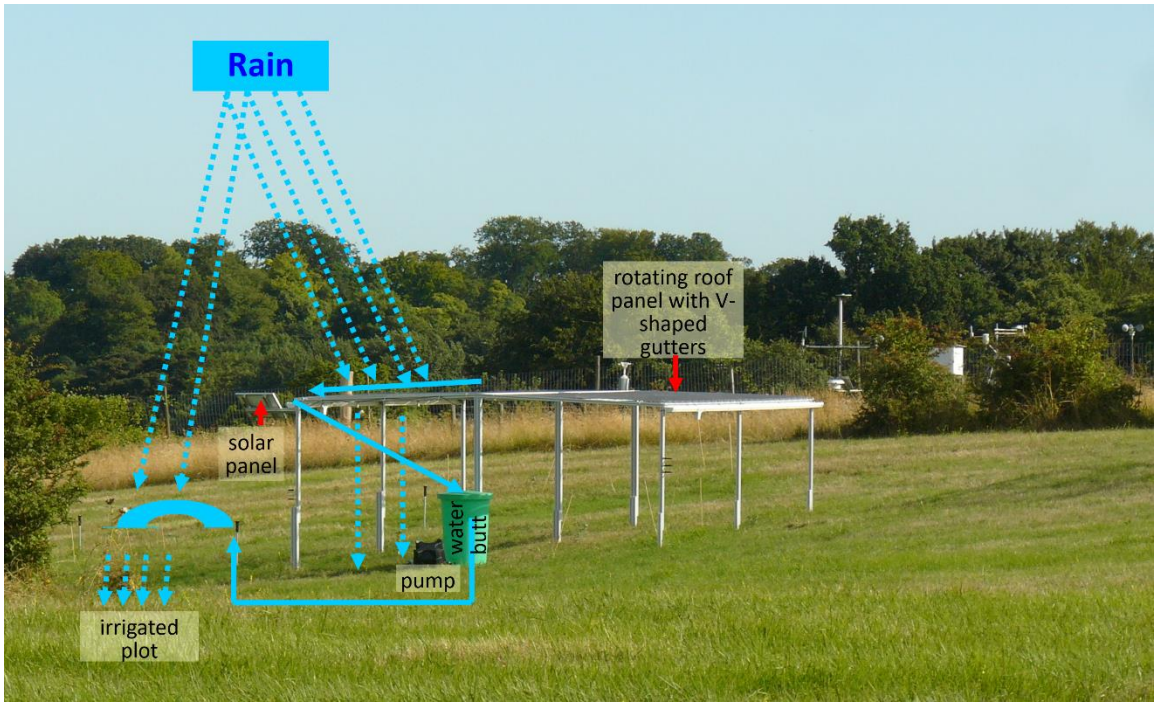


Figure 2.6 Schematic of drought treatment rainshelter and adjacent irrigated plot on Block D, indicating how the RainDrop rainfall collection and redistribution system works.

The rainfall manipulations were operational through the growing season (April to October); outside this time, the rainwater-gathering equipment was removed for storage and maintenance, and interception gutters on drought plots turned over to allow all ambient rainfall through.

2.2.7.2 Sampling layout

Each 5 m x 5 m plot was subdivided to allow nested experimental treatments, and use by Drought Net and other researchers (Figure 2.7).

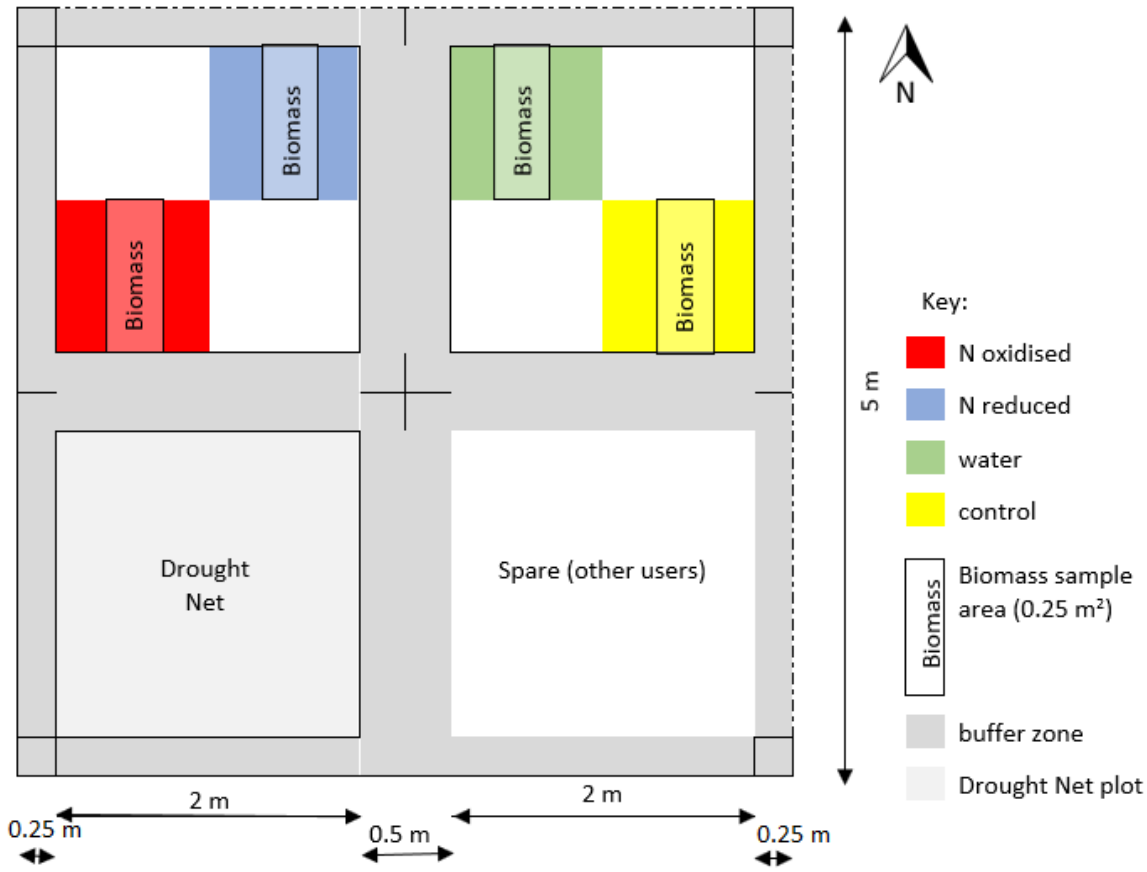


Figure 2.7 Schematic of 5 x 5 m plot subdivisions.

In each plot, the following nitrogen treatments were applied as part of a nested experiment:

- addition of oxidised nitrogen as aqueous $NaNO_3$ (N_{ox})
- addition of reduced nitrogen as aqueous NH_4Cl (N_{red})
- addition of water at same rate as nitrogen treatments (water)
- no additions (control).

In order to prevent quadrats lying adjacent to each other within a quarter-plot, treatments were randomly allocated to diametrically opposed locations. Internal buffer zones prevented quadrats in quarter plots being contiguous.

2.2.7.3 *Verification and adaptation*

The experiment at Upper Seeds used a design of rainshelter based on that used by Yahdjian and Sala (2002) that had not been field-trialled previously, along with an associated irrigation redistribution system. A prototype rainshelter was erected in the grounds of the Open University campus at Walton Hall in 2014, in order to assess whether the design and construction were suitable for deployment in the field. Subsequent monitoring of environmental variables was carried out on site, and findings are dealt with below.

2.2.7.3.1 Effectiveness

The rainshelters were designed to intercept 50% of ambient rainfall, in order to impose partial drought on the underlying experimental plot. When observed during a moderately heavy rain shower, all rain intercepted by the gutters was seen to be successfully routed to the storage tank with no sign of spillage or other loss. Volumetric soil moisture in the top 100 mm was measured using a capacitance probe (Theta Probe, Delta-D Devices, Cambridge), and compared with soil moisture in an adjacent, unsheltered area.

The difference in soil moisture content showed a reduction of 30% when measured at the end of the 2015 growing season; further measurements taken a week later gave a mean reduction in soil moisture beneath the rainshelter of 33% (n=54). Reduced soil moisture corresponded well with the extent of the sheltered area, indicating that rainfall interception had a demonstrable effect on volumetric soil moisture, with a narrow zone of c. 30 cm at the edge where the majority of change in soil moisture occurred (Figure 2.8).

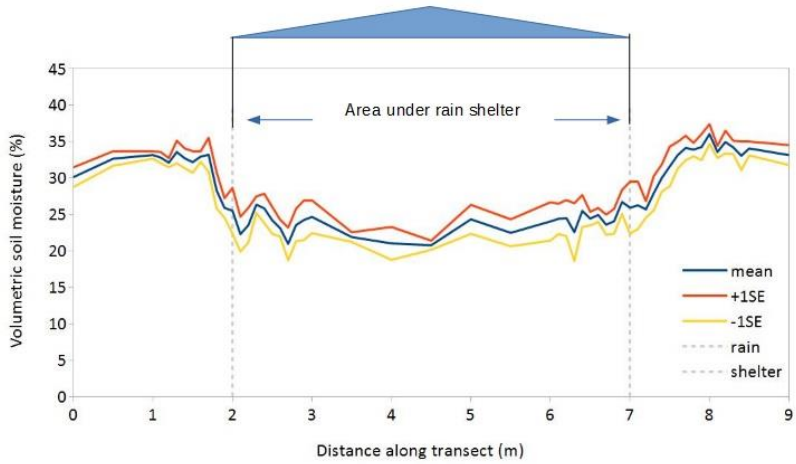


Figure 2.8 Volumetric soil moisture taken along transects across footprint of prototype rainshelter.

Over the summer of 2017, a drought effect was observed under the procedural control shelters, due to precipitation running along the slope of the gutters and falling from the end, i.e. at the limit of the experimental plot. This resulted in reduced productivity in the centre of the plots, and increased plant growth along the east and western plot edges. Volumetric soil moisture measures taken across the plots in August 2017 supported the impression of unexpected drought conditions under the procedural control shelters (Figure 2.9).

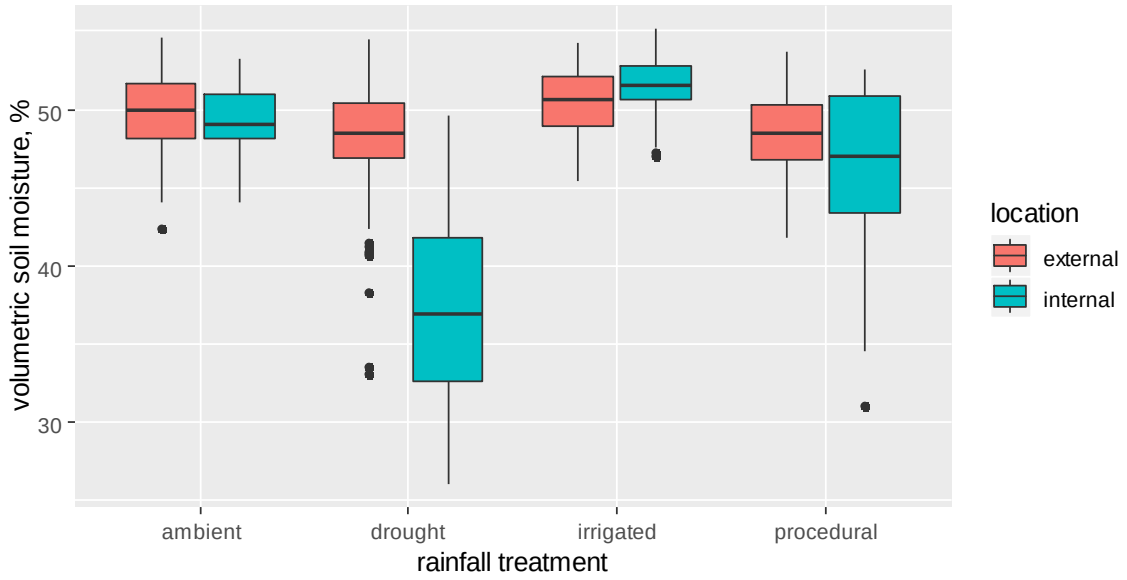


Figure 2.9 Volumetric soil moisture measurements (VSM) taken across treatment plots on 23 August 2017. Internal data were within the 5 x 5 m plot; external data were outside the 5 m plot boundary.

These data, along with the field observations, suggested a design flaw, which was addressed by levelling-up the gutter panels to a horizontal position (rather than being inclined); subsequent observation during rainfall events saw an improvement in rain falling through to the underlying control plot, with raindrop dewpoints being seen along the full length of the downturned gutters.

Soil moisture potential was assessed after the rainfall treatments had been running for over a year; Decagon MPS6 soil water potential sensors were installed at a depth of c. 10 cm in each of the treatments in block E (Decagon Devices, Inc., Pullman WA). Results showed a measurable difference in the soil moisture potential in the four treatments, which confirmed that the rainshelter/redistribution system was altering soil water dynamics (detailed below in Figure 2.10).

Variation in soil moisture potential under the drought treatment was seen to be very responsive to rainfall events, and comparing the sensor outputs with precipitation data from the Wytham T08 AWS confirmed that incidence of rapid lowering of soil moisture potential coincided with precipitation events, e.g. 8 September (16.7 mm), 16 September (19.0 mm), 18 September (7.6 mm), etc).

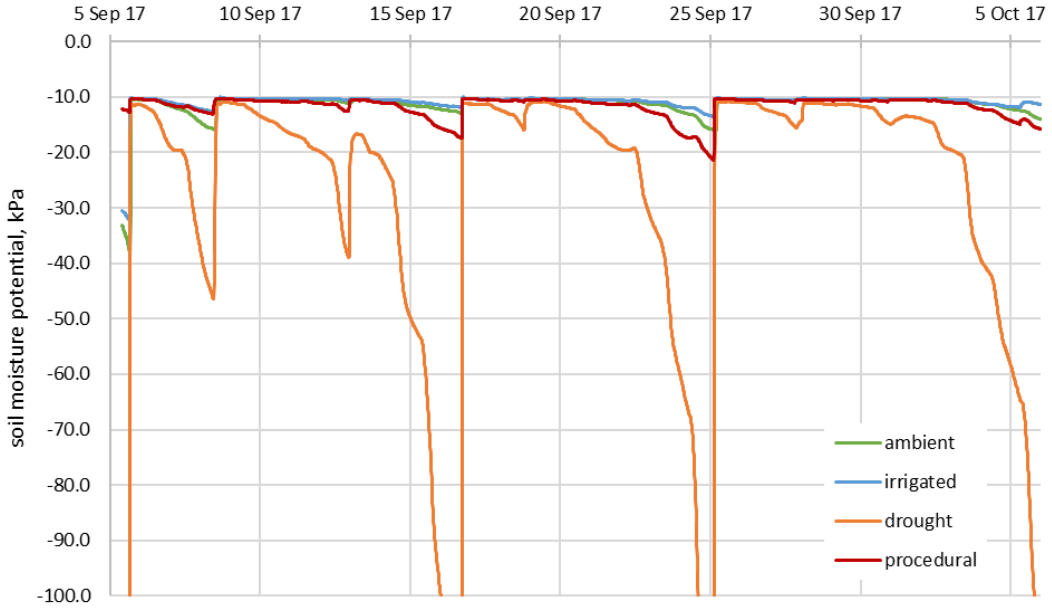


Figure 2.10 Detail of soil moisture potential in the four rainfall treatment plots in block E, September 2017.

Differences in soil VSM between the rainfall treatments were confirmed by ANOVA. Both internal and external soil VSM were found to be significantly influenced by rainfall treatment ($p < 0.001$ for both), and appeared to follow a gradient from wettest (irrigated) to driest (drought) treatment (Table 2.3).

Table 2.3 Mean VSM across rainfall treatment plots in August 2017. Mean VSM is % volume water; grouping is by Tukey's HSD post-hoc test; different group letters indicate significant differences in group means.

	Treatment	mean VSM	Group	Wetness
External to plot	irrigated	50.43	a	wet
	ambient	49.74	ab	↓
	procedural	48.34	bc	
	drought	47.80	c	dry
Internal to plot	irrigated	51.46	a	wet
	ambient	49.22	a	↓
	procedural	46.38	b	
	drought	36.82	c	dry

2.2.7.3.2 Assessment of rainshelter for untargeted effects

Light transmission

The v-shaped gutters on the roof panels were made of Perspex, to allow for the maximal transmission of photosynthetically active (400-700 nm) and infrared radiation. Accumulations of algae and dust in the gutters were found to have a shading effect (18% in bright October sunshine; 11% in neutral shade), which was reduced to 8% by simple washing (Figure 2.11).

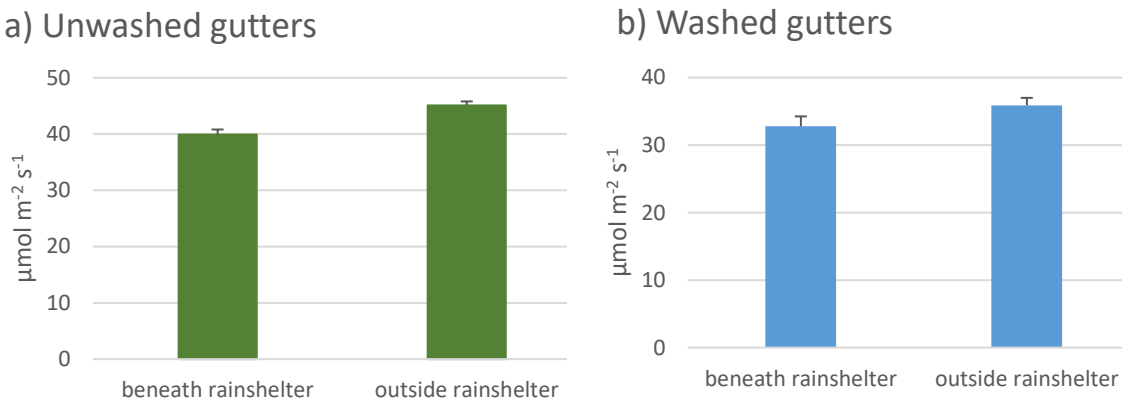


Figure 2.11 Photosynthetically active radiation (PAR) beneath and outside prototype rainshelter in neutral shade/overcast conditions: a) Unwashed gutters; b) washed gutters. Error bars represent one standard error of the mean (n = 10).

Soil and air temperature

Air temperature showed only very minor differences (<0.5 °C) beneath the guttered canopy, with no consistent pattern. Soil temperatures at 2 cm depth were typically 0.2-0.5 °C cooler that soil in a control (unsheltered) area on days with direct sun. Soil temperatures at 10 cm depth showed no measurable effect.

2.2.7.3.3 Irrigation system

The irrigation system was designed so that all upstanding parts could be removed to allow mowing across Upper Seeds (twice annually). It comprised four sprinklers, each operating through a 90° arc, to reserve all irrigation onto the supplemented plots. The distribution of irrigated water was tested on the prototype plot at Walton Hall, with the sprinkler heads mounted on 1 m risers. This found that the central area received a higher proportion of the water, which would result in patchy distribution in the field (Figure 2.12).

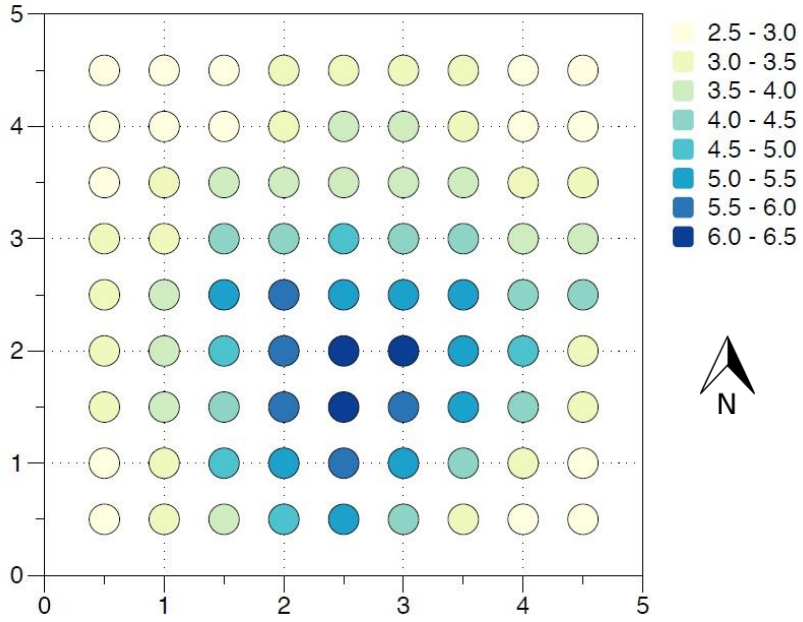


Figure 2.12 Irrigation distribution pattern on test with 1 m risers. The distribution is coded according to the depth of water (cm) in a cup array arranged at intervals of 0.675 m within the 5 x 5 m test plot. The effect of wind blow from the north (top of figure) can be seen in the shift of darker symbols towards the south (bottom of the figure).

A moderately gusty northerly breeze of c. Beaufort 2-3 at the time of the test resulted in the more southerly part of the plot receiving more water due to wind-blow effects. As the sprinkler arc follows a wave pattern, it was felt that reducing the risers to 0.5 m would reduce the overlap sufficiently that lateral movement of water within the soil will even out the distribution, and reduce the amount of misplaced irrigation due to wind blow. To reduce it to less than 0.5 m would have increased the risk of the irrigation being intercepted by tall vegetation, e.g. Wild Parsnip (*Pastinaca sativa*), which is present across much of Upper Seeds.

2.2.7.4 Fitness of hardware for field deployment

The rainshelters succeeded in imposing drought conditions in experimental plots, as indicated by reduced VSM measurements taken at the prototype stage and once installed in the field (e.g. Figure 2.8, Figure 2.9, Table 2.3). The actual proportion of precipitation intercepted or redistributed has not been quantified. It has also not been established how much total precipitation input is lost due to the procedural control shelters; mist, drizzle and light rain may be caught on the gutter surfaces in insufficient volume to generate drops, and so be lost to the plot beneath through evaporation.

2.2.8 Summary of treatments

2.2.8.1 Rainfall

Rainfall treatments were commenced in July 2016, following the baseline species survey and biomass harvest. Thereafter, they were imposed continually from April to October, i.e. for the duration of the growing season (Table 2.4).

Table 2.4 Summary of rainfall treatments.

Treatment	Description	Theoretical % of ambient rainfall
Drought	• 50% of ambient rainfall is intercepted and rerouted away from experimental plot.	50
Irrigated	• receives ambient rainfall, plus the intercepted and rerouted fraction from drought plots.	150
Ambient	• environmental control	100
Procedural	• procedural control – plots with rainshelter structures that allow unimpeded precipitation to pass through to experimental plot.	100

2.2.8.2 Nitrogen

Experimental nitrogen addition treatments were nested within the rainfall treatments, such that each treatment replicate also contained one of each of the four nitrogen treatments (Table 2.5). Nitrogen additions were applied monthly from April to September, for 2017 and 2018, at a monthly equivalent dose rate of 25 kg m^{-2} $year^{-1}$. Doses were prepared from 1M solutions of $NaNO_3$ (Nox) and NH_4Cl (Nred) and delivered in a fixed volume of water (1 litre). The water and no addition ("none") treatments received only ambient background levels of N deposition; the three-year average for total N deposition at the RainDrop site was 19.74 kg N ha^{-1} yr^{-1} (2015-2017) (APIS, 2018).

Table 2.5 Summary of nitrogen addition treatments.

Treatment	Description
Nox	• Oxidised nitrogen as 1M $NaNO_3$ in solution.
Nred	• Reduced nitrogen as 1M NH_4Cl in solution.
water	• Water only (local mains, or rainwater) at same application rate as N solutions, to control for the aqueous component of N solutions.
none	• No additions; control for both N and water additions.

2.2.9 Vegetation investigations - methods

There are several different ways in which community diversity can be measured, such as:

- richness – being the number of individuals exhibiting a particular trait, e.g. species, C-S-R class.
- diversity – which considers both the number of e.g. species present, and their relative abundance ("evenness")
- compositional similarity or dissimilarity

Richness and diversity were assessed on the basis of species, plant groups, life history and C-S-R classes. Community diversity is a function of both number of species present, and their relative abundance; a community dominated by a few species is less diverse than one where species have similar abundance. Simpson's Index of Diversity (D) was calculated for each of the rainfall treatments as follows:

$$D_1 = 1 - \sum_{i=1}^{S} p_i^2$$

where p_i is the proportion of species i and S is the number of species, so that $\sum_{i=1}^{S} p_i = 1$. Values taken by D_1 lie between 0 and 1, where higher values of D_1 indicate higher sample diversity.

Compositional dissimilarity was investigated using the Bray-Curtis dissimilarity index, which takes account of species that two sites or groups of samples have in common, as a function of their total species richness. Bray-Curtis dissimilarity values were calculated to assess species' presence and abundance under the different rainfall treatments, as follows:

$$d_{jk} = \frac{\sum_i |x_{ij} - x_{ik}|}{\sum_i (x_{ij} + x_{ik})}$$

where x_{ij} and x_{ik} refer to the quantity of species i in treatments j and k. Bray-Curtis dissimilarity indices lie between 0 and 1, where 0 indicates that sites share all the same species (i.e. are not at all dissimilar); an index of 1 indicates that the sites have no species in common (i.e. they are totally dissimilar). The Bray Curtis assumption that sites are the same size was met as survey quadrats were all 1 m^2.

Species presence/absence and percentage cover surveys were carried out for each survey quadrat (n = 80), followed as soon as possible by a biomass sampling harvest. The baseline species survey was undertaken in June 2016, prior to the drought and irrigation treatments being activated. Thereafter, further surveys and biomass harvests were taken in June 2017 and June 2018. Throughout, the term "biomass" refers to above-ground biomass only.

Biomass harvests were taken as soon as possible following the species surveys, whereby a strip measuring 0.25 m by 1 m long was cut from each survey quadrat (n = 80). A north-south orientation was imposed to reduce unlooked-for effects of the rain shelter gutters (which are oriented east-west), by cutting across them on the plot below.

In order to assess the amount of biomass this area was supporting, vegetation was parted at ground level and all aerial parts of plants rooted within the clipping zone were harvested. The cut was made at a height of c. 3 cm, to mimic the simulated hay mow that would take place across the whole area as part of the grassland management. Harvested strips were varied in different growing seasons, so each year's harvest was derived from a different 0.25 m^2 strip within each 1 m^2 survey quadrat (Figure 2.13).

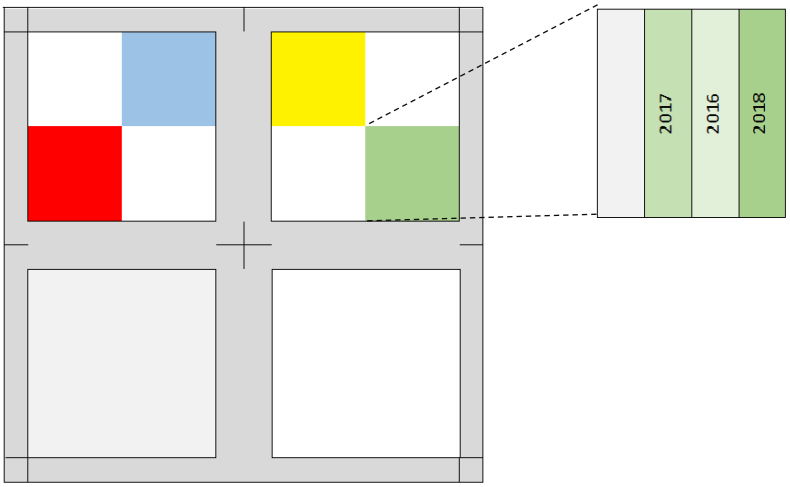


Figure 2.13 Schematic of clipping sequence within a 1 m^2 survey quadrat.

Biomass material was sorted to plant group (graminoids (to include grasses, sedges and wood-rush species); legumes; non-leguminous forbs; woody species; moss; and senesced material (litter)) before being oven-dried at 60 °C, and the resulting dry biomass weighed. Where it was not possible to sort immediately, some samples were stored in deep freeze at -18 °C to prevent decay. Thereafter, they were thawed and treated as fresh material for sorting and drying.

Species identification follows Rose and O'Reilly (2006) and Stace (2010) for forbs, legumes, sedges and woody species; grasses follow Hubbard (1984). Life history data was taken where possible from BRC Atlas of British and Irish Flora (https://www.brc.ac.uk/plantatlas/); where BRC Atlas did not hold this data, other sources were used - Ellenberg Indicator values for *Galium pumilum, Brachypodium pinnatum,* and *Phleum bertolinii*, were taken from the Ecological Flora of the British Isles database (Fitter, A. H. and Peat, H. J., 1994, The Ecological Flora Database, J. Ecol., 82, 415-425. http://ecoflora.org.uk/).

C-S-R strategy data was taken where possible from the UCPE online tool developed by Sheffield University (Hunt *et al.*, 2004); for species not included in UCPE database, other sources were used for C-S-R information, namely Pierce *et al. (*2017) for *Bromus commutatus* and *Vicia sativa*; Dennis (2012) for *Phleum bertolinii*; and *Galium pumilum*, which uses the strategy allocated by Riibak *et al.* (2015). *Ranunculus parvoflora* (assigned R) and *Orobanche minor* (broomrape species, assigned S) were assigned as a result of literature search, though no definitive strategy was found. *Hieracium* species (not including *Pilosella*), *Prunus, Quercus* and *Rosa* species do not have detailed habitat or life form information, as they were only reliably identified to genus.

2.3 Analysis

All analysis was carried out using the R statistical software versions 3.4.1 and 3.6.1 (R Development Core Team, 2018).

Diversity was assessed using Simpson's Index of Diversity for infinite populations,

$$SID = 1 - \sum_{1=1}^{s} p_i^2$$

where p_i is the proportion of species i, and s is the number of species; this was calculated using the diversity() function with option *simpson* in *vegan* package in R (Oksanen, 2015). Plant group diversity was significantly not-normal (by Shapiro Wilks test) for all but one plant group and rainfall treatment combinations, so was analysed with Kruskal Wallis with the pairwise Wilcoxon test used to determine significance of differences between survey years. C-S-R diversity failed Levene's test so was analysed using Kruskal Wallis and pairwise Wilcoxon test as post hoc. Community dissimilarity was investigated using Bray Curtis pairwise comparisons in the vegdist() function in R package *vegan* (Oksanen, 2015).

To investigate whether biomass and abundance responses significantly differed between rainfall and nitrogen treatments and with time, linear mixed-effects models were developed using the lme4 package (Bates *et al.*, 2015), around the following general equation:

$$x \sim F1 + F2 + F1{:}F2 + R1 + R2$$

where x is the response variable, F1 and F2 are fixed factors, and R1 and R2 are random factors. Cover abundance data were log-transformed to improve model fit, using log(x + 1) to enable zero scores to be remapped as zero in the final model. Comparisons of values across groups was carried out using the lsmeans() package (Lenth, 2016).

Where responses from all three years are considered together, the factors take the following identities:

- F1 is a fixed factor with four levels indicating the four rainfall treatments;
- F2 is a fixed factor with four levels, indicating the four nitrogen-addition treatments;

- R1 is a random factor with three levels, included to account for temporal variation across the three sampling years ("date"); and
- R2 is a random factor with five levels to account for spatial variation in the vegetation as captured at the replicate block level on site.

Abundance responses in each experimental year were compared to the 2016 baseline through linear mixed effect models using log response ratio as the response variable, in an amended general equation such that the elements took the following identities:

$$x \sim F1 + F2 + F1{:}F2 + R1$$

- x is log(ratio response), e.g. log(response_year$_b$/response_year$_a$), where year$_a$ is the baseline 2016, and year$_b$ is one of the experiment years.
- F1 as a fixed factor with four levels indicating the four rainfall treatments;
- F2 as a fixed factor with four levels, indicating the four nitrogen-addition treatments;
- R1 as a random factor with five levels to account for spatial variation in the vegetation as captured at the replicate block level on site.

Grass:forbs ratios for individual experimental years were also analysed using the log(response ratio) approach.

Significance of changes in species abundance between 2016-2017 and 2017-2018 were carried out using paired-sample Wilcoxon test (as related to species abundance in the two periods).

Correlations between diversity and biomass were tested using Pearson's product moment where variables passed Shapiro's test, and Spearman rank where they were found to deviate significantly from a normal distribution.

The influence of rainfall treatment on anthill presence/absence was carried out with Mann-Whitney-Wilcoxon ("Mann-Whitney") test (wilcox.test()), to test for association with ambient or imposed environmental conditions. Principal components analysis was carried out on abundance data using singular value decomposition (prcomp() and PCA() in the package *FactoMineR*).

2.4 Results

Plant groups are referred to as follows:

- **grass**: all graminoids, including grasses, sedges and wood rush species
- **forb**: all non-leguminous or non-woody forbs
- **legume**: all legume species
- **woody**: all tree species; also *Rubus* and *Rosa* species
- **moss**: all moss species were amalgamated to give total abundance for moss

Data were also gathered on other ground cover types:

- **litter**: senesced ("dead") material laying on ground surface
- **bare**: the area of bare soil surface visible within the survey quadrat.

Species list and information can be found in Appendix 2.

Results pertinent to individual hypotheses can be found as follows:

• H2.1: Diversity will show a negative correlation with received rainfall amount, i.e. will be higher under the drought treatment	*in section*	2.4.1
• H2.2: Grass to forb abundance ratio will increase under enhanced rainfall treatment compared to the drought treatment	*in section*	2.4.4.2.4
• H2.3: More competitive species will increase in abundance under enhanced rainfall compared to the drought treatment	*in section*	2.4.4.4
• H2.4: Reduction in above-ground biomass under the drought treatment will be proportionally similar to an increase in above-ground biomass under the enhanced rainfall treatment	*in section*	2.4.5.3
• H2.5: Plot biomass will increase in response to nitrogen addition, where moisture availability is sufficient. Where soil moisture is insufficient for nitrogen-uptake, nitrogen addition will have no effect on plot biomass	*in section*	2.4.5.2

2.4.1 Diversity measures

Associated hypothesis:

- H2.1: Diversity will show a negative correlation with received rainfall amount, i.e. will be higher under the drought treatment.

2.4.1.1 Richness

A total of 104 plant species were observed within the survey quadrats over the course of the species surveys:

- 29 grasses, sedges and wood-rushes (5 annual, 24 perennial)
- 58 forbs (11 annual, 3 biennial, 43 perennial, and one unknown (broomrape species))
- 9 legumes (4 annual, 5 perennial)
- 8 woody (all perennial; 6 of these were tree species, and mainly seedlings).
- Only one species of moss has so far been identified – *Rhytidiadelphus squarrosus*. This is a very common moss of unimproved or semi-improved grassland.

Species abundance summaries can be found in Appendix 2.3.

Species richness within the survey quadrats was reduced in 2017 (72 species) compared to 2016 and 2018 (both 84 species), this reduction in species number was spread across all plant groups (Table 2.6) and rainfall treatments (Table 2.7).

Table 2.6 Annual summaries of species and plant group richness for midseason surveys.

	year			total across all 3 years
number of...	2016	2017	2018	
species	84	72	84	104
graminoids	23	18	23	29
forbs	45	41	48	58
legumes	9	7	7	9
woody	4	3	5	8

Table 2.7 Species richness within rainfall treatments, at midseason surveys (June).

	rainfall treatment				total number of species
year	ambient	irrigated	drought	procedural	
2016	62	61	58	68	84
2017	57	56	56	57	72
2018	60	65	62	65	84

The 100 species for which C-S-R information was available were represented by 17 C-S-R classes (Figure 2.14):

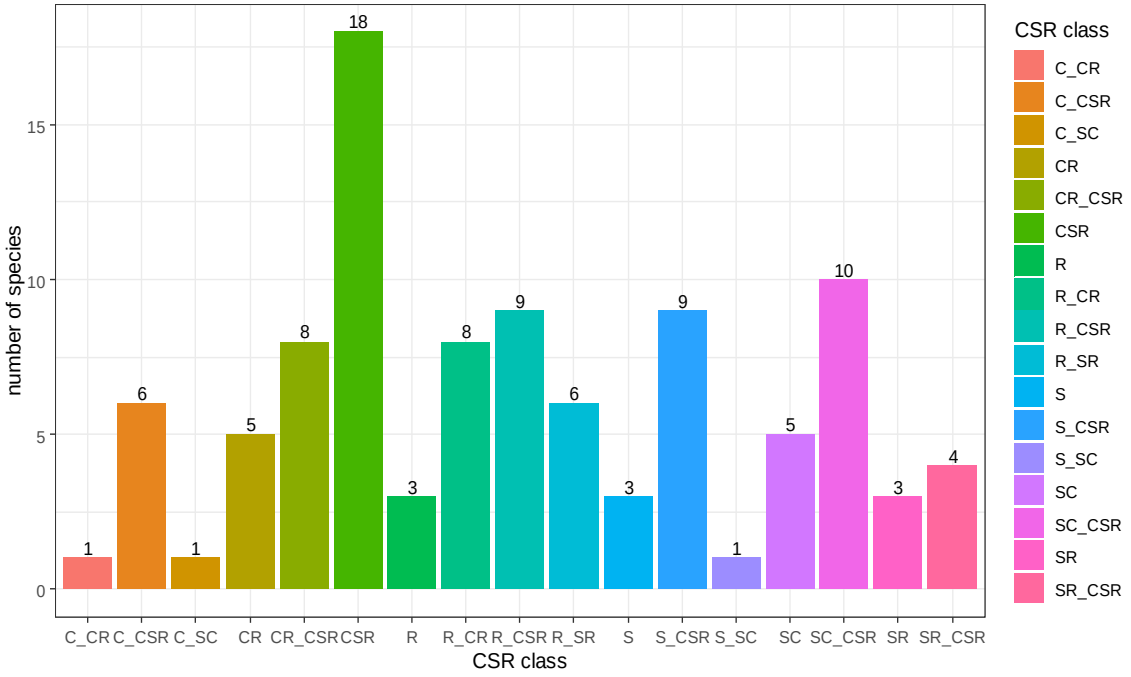


Figure 2.14 Number of species in each C-S-R class.

All 17 C-S-R classes were present in 2016, reducing to 15 in 2017 and 16 in 2018. The three ruderal (class R) species were not observed in any quadrats in June 2017 (being *Lamium purpureum*, *Ranunculus parviflorus* and *Poa annua*), nor was *Anthriscus sylvestris*, the only member of the C/CR class, which did not return into survey quadrats in 2018 either.

In order to simplify analysis, the 17 C-S-R classes were aggregated into seven secondary classes, all of which were present on site in all treatments in all three surveyed years. Further details can be found in Appendix 2.

2.4.1.2 Species diversity

Simpson's Index of Diversity was comparable across all treatment plots prior to the treatments being initiated; thereafter, species diversity was higher in drought plots and lower in procedural control plots, compared with the irrigated and ambient control plots, which were equivalent (Figure 2.15).

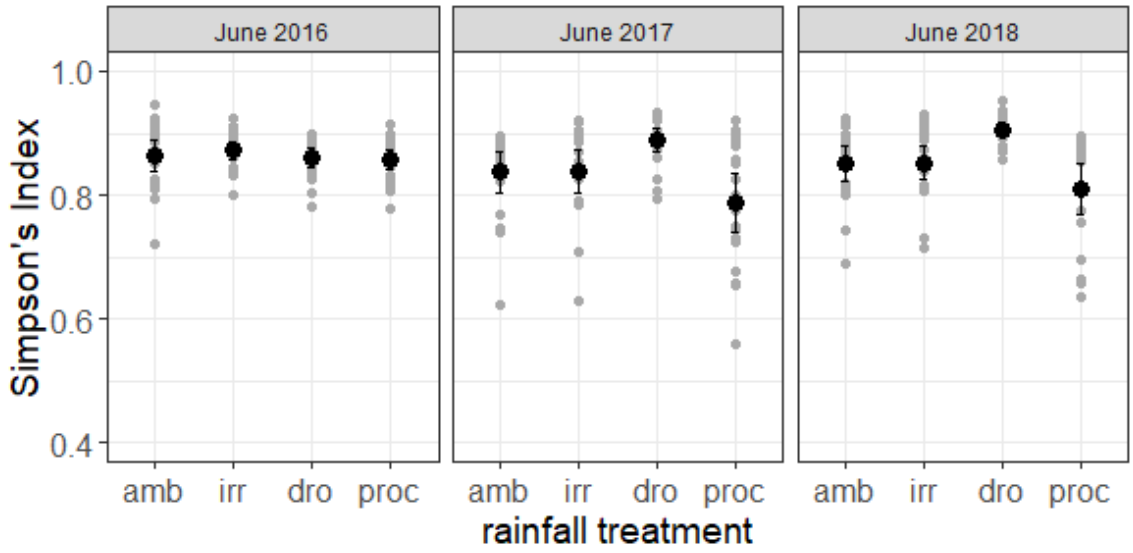


Figure 2.15 Simpson's Index of Diversity for the rainfall treatments: higher values indicate higher diversity. Grey dots are data points, black dots are means; error bars are ±1 SE. Key to x-axis: amb = ambient control, irr = irrigated plots, dro = drought plots, proc = procedural control.

The disparity between diversity of drought and procedural control plots was explained by the dominance of a few species under the procedural controls, where nearly 50% of cover from grass, forb, legume and woody species was provided by only four species in both 2017 and 2018 (*Lotus corniculatus* 23%, *Arrhenatherum elatius* 12.5%, *Brachypodium pinnatum* and *Trifolium repens* both 7% in 2017; *Lotus corniculatus* 23%, *Arrhenatherum elatius* 13%, *Trifolium repens* 10% and *Medicago lupulina* 7% in 2018).

In contrast, abundance of species in drought plots was more even, requiring seven species to provide 50% of cover, with *Arrhenatherum elatius* (11%), *Galium verum* (9%), *Trifolium repens* (7.5%), *Potentilla reptans* (7%), *Brachypodium pinnatum* (6%) and *Clinopodium vulgare* (6%) and *Pastinaca sativa* (5%) being most abundant in 2017 (all other species contributing less than 5% each). In 2018, *Galium verum* and *Pastinaca sativa* were replaced by *Medicago lupulina* and *Lotus corniculatus* as more abundant species. Moss cover, percentage cover of litter and bare ground were also greater under drought shelters than in procedural control plots. The

combined abundance of grass, forb and legume plant groups was greater under procedural controls in both 2017 and 2018.

2.4.1.3 Plant group diversity

Plant group diversity in the survey quadrats was seen to vary year on year (Figure 2.16), reflecting changes in abundance of dominant species (see Table 2.8 for most abundant species in each year).

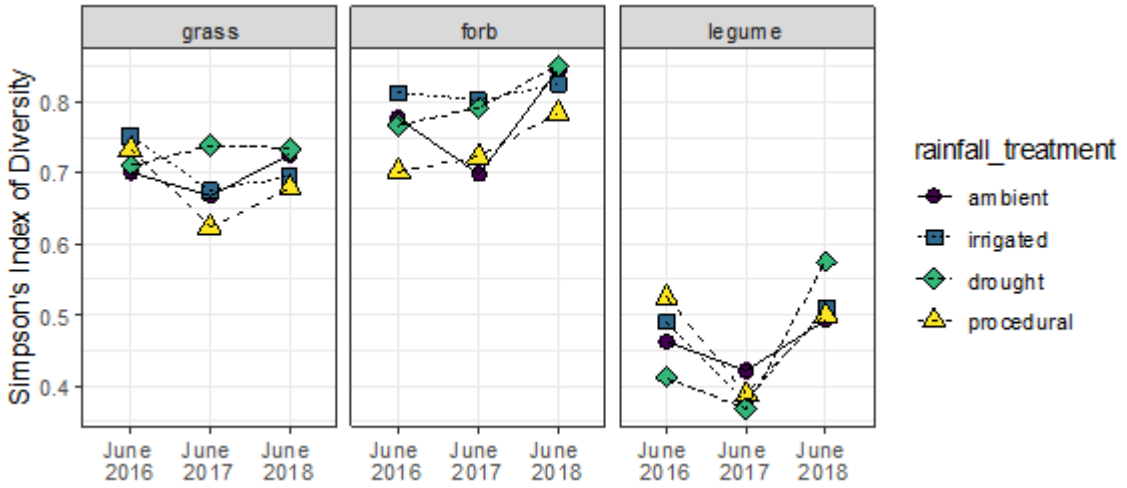


Figure 2.16 Plant group diversity indices for grasses, forbs and legumes at midsummer for 2016 (baseline), 2017 and 2018. Higher values of Simpson's Index indicate higher diversity.

The effect of rainfall treatment on grass diversity in the survey quadrats was only significant for the decline in diversity between 2016 and 2017 under the procedural control shelters ($t_{(19)}$ = 3.2777, p = 0.0040**). Grass diversity increased under the drought treatment, and maintained a higher value of Simpson's Index of Diversity in both 2017 and 2018; both drought and ambient control plots had a small net increase in grass diversity 2016-2018. Grass diversity in all other treatments mapped similar patterns of variation between years, with a decline in diversity in 2017 followed by a relative increase in 2018. Grass diversity in irrigated and procedural control plots did not return to the baseline level in 2018, suggesting that some species had declined in abundance or been lost from these communities, and that the grass portion of the community was becoming dominated by fewer species in those treatment plots.

Forb diversity increased in association with the presence of rainshelters, i.e. both drought and procedural control plots became more diverse in both years following the start of the treatments. By 2018, diversity under the droughted plots was significantly higher than it had been prior to the shelters being installed ($t_{(19)}$ = -3.005, p = 0.0073**). There was least change in

diversity in the irrigated plots, even in the dry summer of 2017, suggesting that increased soil moisture availability was acting to stabilise forb diversity. Forb diversity in the ambient control plots was significantly higher in 2018 than in 2017 ($t_{(19)}$ = -3.4343, p = 0.0028**).

Legume diversity closely followed mean Spring rainfall, and was reduced in 2017 following the dry spring and early summer of that year, compared with both the baseline and 2018 diversity indices. Diversity was significantly higher in 2018 in drought plots than in either 2016 or 2017 (2016-2018 $t_{(19)}$ = -3.1805, p = 0.0049**; 2017-2018 $t_{(19)}$ = -3.5627, p = 0.0021**). There was no net significant change in legume diversity in the other three treatments over the two experiment years.

2.4.1.4 C-S-R diversity

C-S-R diversity was investigated using Simpson's Index of Diversity (Figure 2.17). No significant changes were detected in C-S-R diversity in the irrigated or ambient control plots.

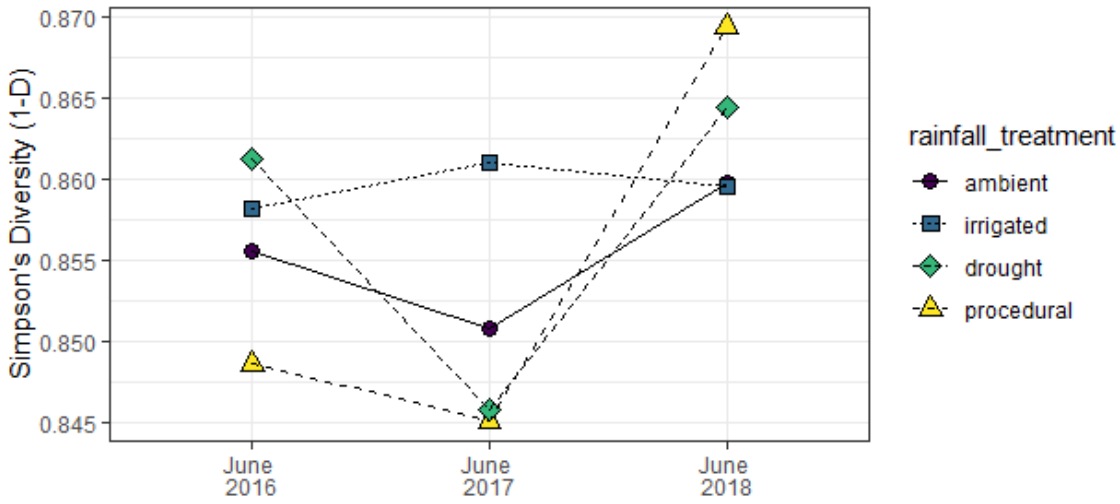


Figure 2.17 C-S-R diversity at pre-treatment baseline (2016) and midsummer 2017 and 2018.

Net C-S-R diversity increased significantly only under the procedural control shelters between 2016 and 2018 ($t_{(19)}$ = -3.2448, p = 0.0043**). C-S-R diversity fell in 2017 in all but the irrigated plots, which saw a slight increase in diversity in that year; in the drought plots, this reduction was significant ($t_{(19)}$ = -2.1537, p = 0.0443*). Both sheltered plots (drought and procedural control) saw a significant increase in C-S-R diversity from 2017 to 2018 (drought: $t_{(19)}$ = -2.5288, p = 0.0205*; procedural control: $t_{(19)}$ = -2.7001, p = 0.0142*). For the drought plots, this returned the diversity measure close to its starting point in 2016; the procedural control plots, however, saw a significant net increase in C-S-R diversity compared to the 2016 level, and the

previous year, 2017. C-S-R diversity was not found to be correlated with quadrat biomass, in any year.

2.4.2 Changes in community membership and composition

2.4.2.1 Community dissimilarity

Quadrats were most dissimilar in 2017 across all rainfall treatments (Figure 2.18); the spring and early summer rainfall that year were low and an early-season drought was considered the explanation for declines in drought-intolerant species, while the number of ruderals present was also low compared with 2016 and 2018.

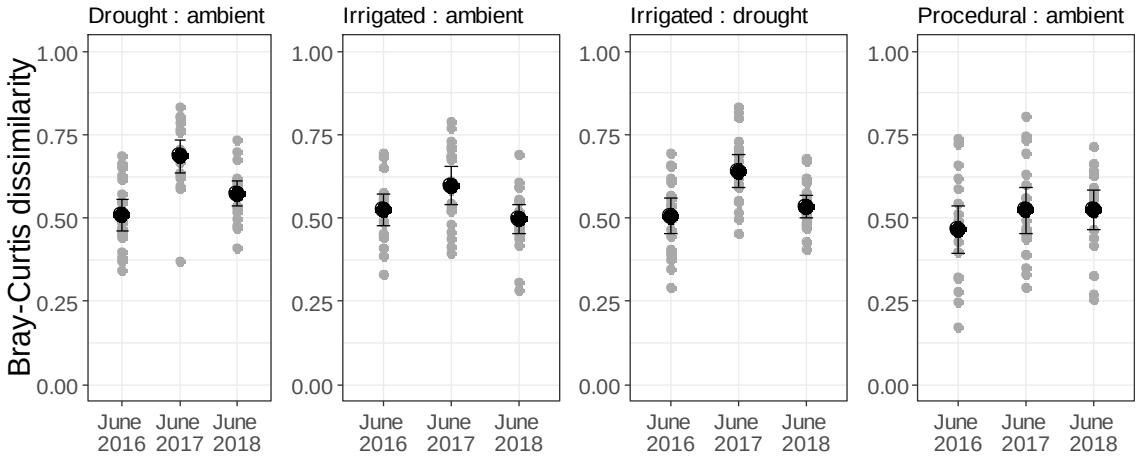


Figure 2.18 Bray-Curtis dissimilarity for all rainfall treatments from 2016 to 2018. Where value = 0, sites share all the same species; where value = 1, sites have no species in common. Higher values for Bray-Curtis mean that sites are more dissimilar.

Data generally supported the hypothesis (H2.1) that diversity would be higher in droughted plots compared to the irrigated plots. This held for species diversity in both 2017 and 2018; all plant groups in 2018, and grass group in 2017; and C-S-R groups in 2018.

2.4.2.2 Species composition

Across the three summer surveys, cover was dominated by only a few species, with a fairly consistent number of species accounting for both up to 50% (five or six species) (Table 2.8) and 75% of all the cover in the survey quadrats, although the most abundant species' identity varied year on year. In all three years, the ten most abundant species accounted for over 60% of total plant cover (62.9% in 2016; 66.6% in 2017; 69.6% in 2018).

Summed cover for species present was highest in irrigated plots, in 2017 and 2018, suggesting that the vegetation structure in these plots was more complex than in the other treatment plots, and individual plants were able to actively compete for light through expansion in vertical niche space.

Table 2.8 Most abundant species, accounting for c. 50% of total surveyed cover across all quadrats.

	species	species cover (m^2)	mean cover (m^2)	no. of quadrats	% of total cover
2016	*Trisetum flavescens*	21.20	0.27	79	20.0
	Clinopodium vulgare	8.88	0.12	75	8.6
	Arrhenatherum elatius	6.17	0.12	51	5.9
	Lotus corniculatus	5.85	0.19	31	5.6
	Festuca rubra	5.09	0.09	55	4.9
	Trifolium repens	4.36	0.06	68	4.2
2017	*Lotus corniculatus*	9.55	0.21	45	12.2
	Arrhenatherum elatius	9.44	0.12	76	12.1
	Brachypodium pinnatum	7.75	0.15	51	9.9
	Trifolium repens	6.00	0.09	70	7.7
	Potentilla reptans	4.64	0.08	62	5.9
	Clinopodium vulgare	3.78	0.06	69	4.8
2018	*Trifolium repens*	11.83	0.15	80	13.7
	Lotus corniculatus	10.20	0.18	57	11.8
	Arrhenatherum elatius	9.38	0.12	77	10.9
	Brachypodium pinnatum	7.48	0.13	59	8.7
	Medicago lupulina	6.56	0.08	78	7.6

2.4.3 Underlying heterogeneity

Over the three survey years, cover classes were found to have differing temporal and spatial distributions (Figure 2.19), which were assessed using mixed effect linear models.

Date of survey accounted for more variation in the data for grass, legume, moss, litter and bare ground cover classes, compared with variation due to block, suggesting that distributions across the site were more fluid in response to ambient conditions in each year than to spatial variation across the site (Table 2.9). Variation attributed to block as a random effect was greater than

variation due to date for forb cover, suggesting that forb cover maintained a core range throughout the three years.

Table 2.9 Variance attributed to random effects (block, date) and residuals from linear mixed-effect models run on cover class abundance data in all years.

cover class	variance attributed to			% of total variance	
	block	date	residual	block	date
grass	0.0147	0.0860	0.1309	6.3	37.1
forb	0.0368	0.0048	0.1600	18.3	2.4
legume	0.0571	0.1528	0.1417	16.7	43.2
moss	0.0003	0.0010	0.0078	3.2	11.3
litter	0.0963	0.1571	0.5431	12.1	19.7
bare	0.0329	0.1420	0.5219	4.7	20.0

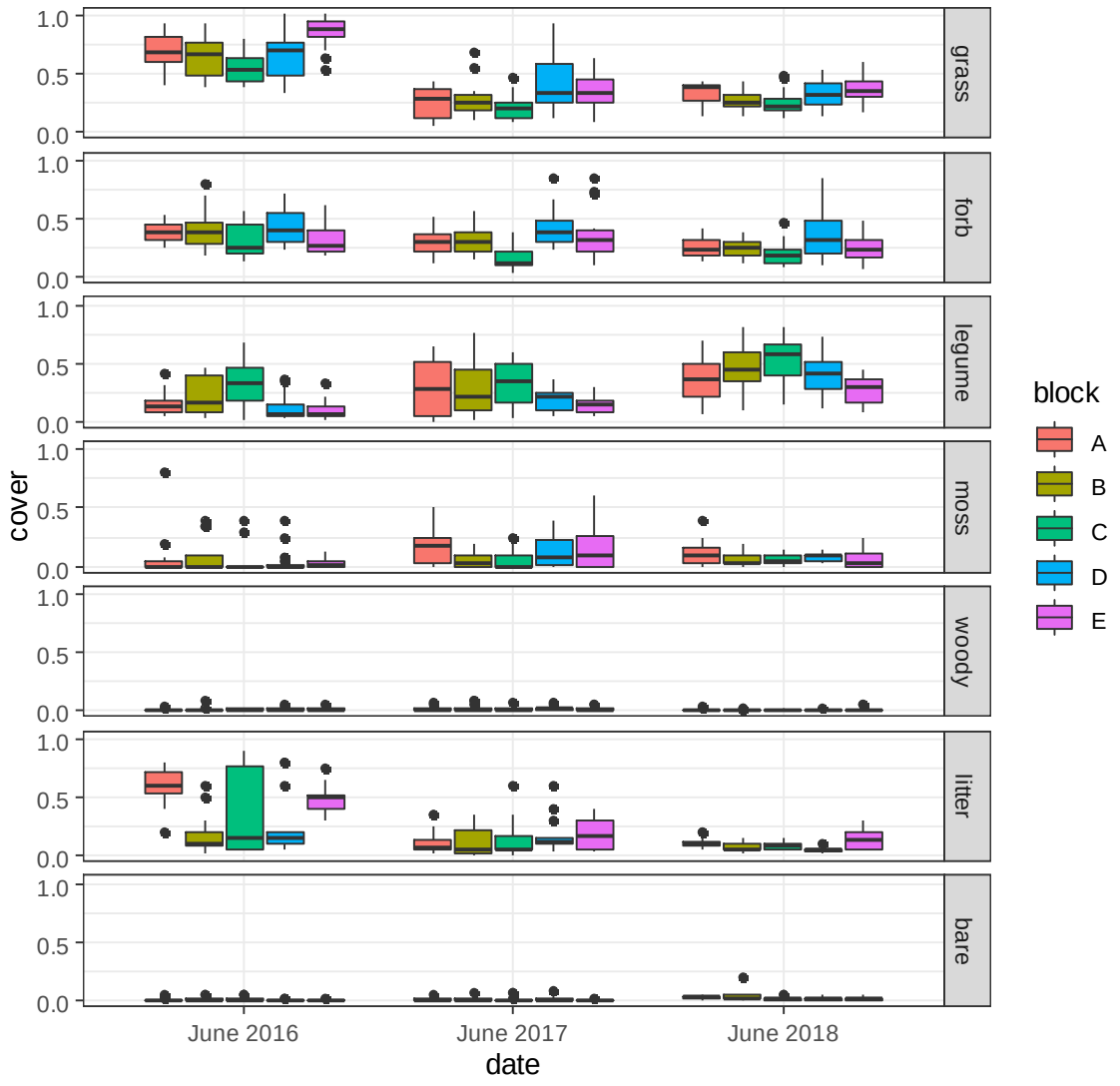


Figure 2.19 Cover class distribution through time (date) and space (block).

Spatial heterogeneity of the vegetation across the RainDrop site at Upper Seeds was captured in the baseline surveys and first biomass harvest (both June 2016). PCA of species' abundance data (measured as % cover) at the baseline survey showed variation with block as a factor (Figure 2.20.

PC1 and PC2 appear to be associated with the location of the blocks themselves within the site; PC1was interpreted as being latitude, running from north at the left (negative values) to south at the right (positive values), as blocks B and C are the most northerly.

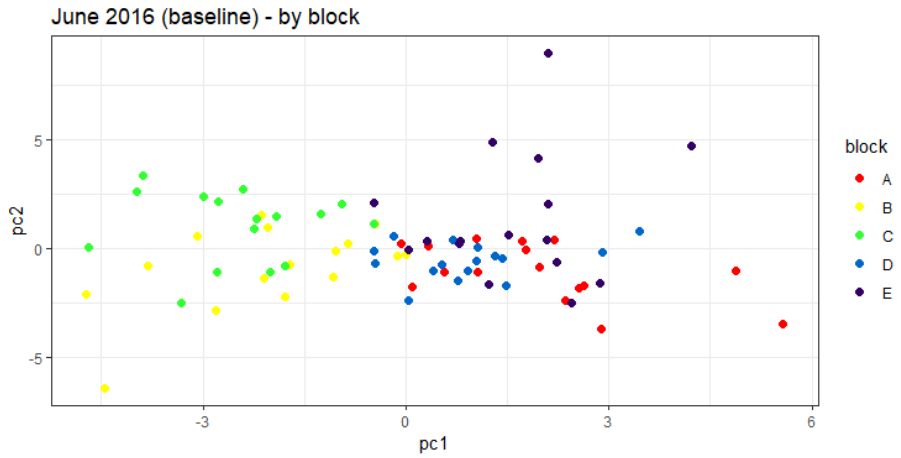


Figure 2.20 Survey 1 (baseline, June 2016) - principal components PC1 and PC2 for cover data. Note differentiation of blocks B and C from the other three.

By June 2018, the plots had had two years of imposed rainfall treatments, and one year of N-addition treatments, and there has been a negative shift along PC1 as a result, with less differentiation between individual blocks (Figure 2.21).

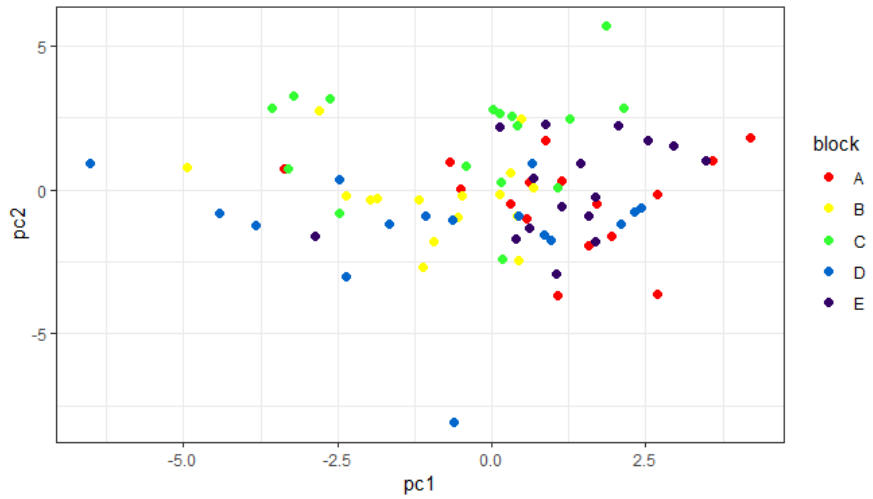


Figure 2.21 Survey 5 (June 2018) - principal components PC1 and PC2 for cover data.

The vegetation in block C was less productive than that in other blocks at the outset of the experiment (mean quadrat total biomass, $\chi^2 = 91.94$, 4 df, $p < 2.2 \times 10^{-16}$) (Figure 2.22).

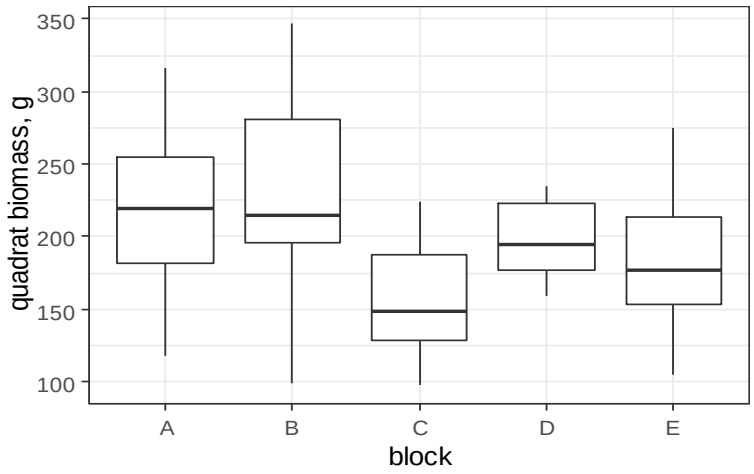


Figure 2.22 Mean quadrat biomass by block, from baseline harvest (June 2016).

This block effect was considered to be an indication of variation in unmeasured aspects of the site, such as soil depth, past land use and disturbance history, and micro-habitat formation due to topographic heterogeneity. In order to account for underlying spatial variation as reflected in the vegetation, block was included as a random effect in subsequent analysis using linear mixed effects models.

2.4.4 Abundance

Associated hypotheses:

- H2.2: Grass to forb abundance ratio will increase under enhanced rainfall treatment compared to the drought treatment.

- H2.3: More competitive species will increase in abundance under enhanced rainfall compared to the drought treatment.

Abundance was measured as aerial cover.

2.4.4.1 Species abundance

When abundance was tested with linear mixed effect models (of format "abundance ~ rainfall treatment * N treatment + baseline as covariate + block as random effect), five species were

found to be significantly influenced by the treatment factors and a further eight species were identified with significant pairwise contrasts between treatment combinations when species abundance in 2017 and 2018 was assessed in comparison to baseline (2016) data. the time of the June 2017 or June 2018 surveys, i.e. after one or two years of treatment. Of these, four were graminoids, five were forbs and four were legume species (Table 2.10).

Table 2.10 Significant response of species abundance to rainfall and nitrogen treatments, as identified by linear mixed effects models.

model						
factor	**estimate**	**SE**	**df**	**t-value**	**p**	
***Brachypodium sylvaticum* abundance 2017 ~ F1 + F2 + F1:F2 + C1 + random**						
irrigated:Nox	0.1117	0.0338	59.62	3.310	0.0016	**
Significant pairwise comparisons						
comparison		estimate	SE	df	t-ratio	p
irrigated – procedural		0.0361	0.0120	59.5	3.013	0.0193
***Bromus hordeaceus* abundance 2017 ~ F1 + F2 + F1:F2 + C1 + random**						
Significant pairwise comparisons						
comparison		estimate	SE	df	t-ratio	p
ambient – irrigated		-0.0115	0.0040	59.0	-2.862	0.0289
irrigated – procedural		0.0114	0.0041	59.1	2.774	0.0362
***Clinopodium vulgare* abundance 2018 ~ F1 + F2 + F1:F2 + C1 + random**						
Significant pairwise comparisons						
comparison		estimate	SE	df	t-ratio	p
ambient –drought		-0.0295	0.0101	59.2	-2.939	0.0236
irrigated – drought		-0.0285	0.0104	59.9	-2.750	0.0383
***Geranium columbinum* abundance 2017 ~ F1 + F2 + F1:F2 + C1 + random**						
Significant pairwise comparisons						
comparison		estimate	SE	df	t-ratio	p
ambient – drought		0.0045	0.0015	59.3	3.005	0.0198
irrigated – drought		0.0045	0.0015	59.1	3.019	0.0190
***Glechoma hederacea* abundance 2018 ~ F1 + F2 + F1:F2 + C1 + random**						
Nox	-0.0056	0.0026	59.08	-2.114	0.0388	*
irrigated : Nox	0.0096	0.0037	59.04	2.582	0.0123	*
procedural : Nred	0.0076	0.0037	59.04	2.041	0.0457	*

Significant pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
ambient – drought	-0.0038	0.0013	59.2	-2.891	0.0268	
irrigated – drought	-0.0042	0.0013	59.2	-3.137	0.0138	
procedural – drought	-0.0043	0.0013	59.1	-3.255	0.0099	
***Holcus lanatus* abundance ~ F1 + F2 + F1:F2 + C1 + random**						
Significant pairwise comparisons 2017						
comparison	estimate	SE	df	t-ratio	p	
irrigated – procedural	0.0270	0.0086	59.3	3.141	0.0136	
irrigated – drought	0.0278	0.0085	59.0	3.263	0.0097	
Significant pairwise comparisons 2018						
comparison	estimate	SE	df	t-ratio	p	
ambient – procedural	0.0300	0.0070	59.3	4.270	0.0004	
ambient – drought	0.0251	0.0069	59.1	3.626	0.0033	
irrigated – procedural	0.0197	0.0070	59.2	2.828	0.0316	
***Lolium perenne* abundance 2018 ~ F1 + F2 + F1:F2 + C1 + random**						
Significant pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
ambient – irrigated	-0.0121	0.0043	59.1	-2.841	0.0305	
irrigated – procedural	0.0161	0.0043	59.0	3.778	0.0020	
Nox – Nred	0.0120	0.0043	59.0	2.814	0.0327	
***Lotus corniculatus* abundance 2017 ~ F1 + F2 + F1:F2 + C1 + random**						
procedural – water	0.1783	0.0879	59.23	2.029	0.0469	*
***Lotus corniculatus* abundance 2018 ~ F1 + F2 + F1:F2 + C1 + random**						
water	-0.1358	0.0650	58.83	-2.091	0.0409	*
irrigated – water	0.2034	0.0918	58.81	2.215	0.0306	*
procedural – water	0.2010	0.0920	58.92	2.184	0.0330	*
Significant pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
ambient – procedural	-0.0870	0.0328	59.4	-2.655	0.0487	
***Medicago lupulina* abundance 2017 ~ F1 + F2 + F1:F2 + C1 + random**						
procedural – water	0.1783	0.0879	59.23	2.029	0.0469	*
***Medicago lupulina* abundance 2018 ~ F1 + F2 + F1:F2 + C1 + random**						

water	-0.1358	0.0651	58.83	-2.091	0.0409	*
irrigated – water	0.2034	0.0918	58.81	2.215	0.0306	*
procedural – water	0.2010	00920	58.92	2.184	0.0330	*
Significant pairwise comparisons						
comparison		estimate	SE	df	t-ratio	p
ambient – procedural		-0.0870	0.0328	58.92	2.184	0.0330
***Potentilla reptans* abundance ~ F1 + F2 + F1:F2 + C1 + random**						
Significant pairwise comparisons 2017						
comparison		estimate	SE	df	t-ratio	p
ambient – irrigated		0.0664	0.0232	59.0	2.857	0.0293
ambient – procedural		0.0797	0.0233	59.1	3.422	0.0061
ambient – drought		0.0714	0.0234	59.1	3.050	0.0175
Significant pairwise comparisons 2018						
comparison		estimate	SE	df	t-ratio	p
procedural – drought		-0.0253	0.0146	59.8	-3.401	0.0064
***Trifolium repens* abundance 2018 ~ F1 + F2 + F1:F2 + C1 + random**						
Significant pairwise comparisons						
comparison		estimate	SE	df	t-ratio	p
ambient – drought		0.1299	0.0382	59.0	3.403	0.0064
irrigated – procedural		0.1349	0.0382	59.0	3.529	0.0044
irrigated – drought		0.2145	0.0402	59.2	5.339	<0.0001
***Vicia sativa* abundance 2017 ~ F1 + F2 + F1:F2 + C1 + random**						
irrigated	0.0060	0.0025	59.12	2.432	0.0181	*
Significant pairwise comparisons						
comparison		estimate	SE	df	t-ratio	p
irrigated – procedural		0.0035	0.0012	59.2	2.838	0.0307
irrigated – drought		0.0040	0.0012	59.0	3.268	0.0095
***Viola hirta* abundance 2017 ~ F1 + F2 + F1:F2 + C1 + random**						
Nred	0.0040	0.0016	63.00	2.554	0.0130	*
drought – Nox	-0.0048	0.0022	63.00	-2.123	0.0377	*
drought – Nred	-0.0060	0.0022	63.00	-2.709	0.0087	**

There was a high degree of natural heterogeneity in the vegetation across the site, which was captured as apparent associations between some species and some experimental plots in the June 2016 survey (Table 2.11).

Eight species showed an apparent association with rainfall treatment only in 2016 (*Agrostis capillaris*, *Anthriscus sylvestris*, *Carex flacca*, *Leontodon autumnalis*, *Prunella vulgaris*, *Sherardia arvensis*, *Taraxacum* agg. and *Trifolium campestre*); as the June 2016 survey took place before the treatments were initiated, this apparent association was interpreted as being indicative of strongly localised distribution on site, as part of the overall vegetation heterogeneity. They are included here for completeness, and also because they may indicate a historical persistence of localised distribution for some species across the site, that would not be fully erased due to the community responses to the treatments. For example, *Lotus corniculatus* (a perennial legume), showed significant differences in cover values associated with rainfall treatment plots in all three midseason surveys, which may indicate a highly clumped distribution on site as well as, or in place of, being a true response to the changing moisture levels under the rainfall treatments.

*Table 2.11 Species that had a weak ($p < 0.1$) or significant ($p < 0.05$) abundance response to rainfall and nitrogen addition treatments. Species with a response only in 2016 are included for historical value and to help identify pre-existing patterns of distribution. Key: rain = rainfall treatment, N = nitrogen addition treatment (either form), rain:N = interaction between rainfall and nitrogen addition treatments (2017 only). Significance levels are indicated as follows: ^ $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

		June 2016		June 2017			June 2018	
species	**plant group**	**rain**	**N**	**rain**	**N**	**rain:N**	**rain**	**N**
Agrimonia eupatoria	forb						**	
Agrostis capillaris	grass	*						
Anthriscus sylvestris	forb	^						
Brachypodium pinnatum	grass			^				
Brachypodium sylvaticum	grass			**	^	*		
Bromus commutatus	grass						^	
Bromus hordeaceus	grass			*				
Carex flacca	grass	**						
Cerastium fontanum	forb				^			
Clinopodium vulgare	forb						**	
Convolvulus arvensis	forb			^				
Dactylis glomerata	grass	*		^			^	
Festuca rubra	grass						^	

Table 2.11 *continued over ...*

... Table 2.11 *continued*

species	plant group	June 2016		June 2017			June 2018	
		rain	N	rain	N	rain:N	rain	N
Galium verum	forb		^				^	
Geranium columbinum	forb			*			^	
Geranium dissectum	forb	**						^
Glechoma hederacea	forb	*					**	
Holcus lanatus	grass			**			***	
Leontodon autumnalis	forb	*						
Lolium perenne	grass			^			**	*
Lotus corniculatus	legume	*		**			**	
Medicago lupulina	legume	^		^			*	
Potentilla reptans	forb			*			**	
Prunella vulgaris	forb	^						
Rubus fruticosus	woody	***		*				
Senecio erucifolius	forb				^			
Sherardia arvensis	forb		*					
Taraxacum agg.	forb	*						
Trifolium campestre	legume	*						
Trifolium repens	legume	*					***	
Vicia hirsuta	legume						^	^
Vicia sativa	legume			*				
Viola hirta	forb	^		*				

Net changes in species' abundance within the treatment plots after two years of imposed rainfall manipulation were greatest in grass and legume species (Table 2.12, Table 2.13), whereas species with decreased abundance (Table 2.14, Table 2.15) included grasses and forbs.

Table 2.12 Highest net area cover increases in species abundance, 2016-2018.

plant group	species	rainfall treatment	area difference (m^2)	proportion difference
legume	*Trifolium repens*	irrigated	+ 4.28	+ 8.25
legume	*Lotus corniculatus*	procedural	+ 2.35	+ 1.87
legume	*Trifolium repens*	ambient	+ 2.19	+ 2.55
legume	*Medicago lupulina*	irrigated	+ 1.95	+ 6.57
grass	*Brachypodium pinnatum*	drought	+ 1.82	+ 7.07
grass	*Arrhenatherum elatius*	procedural	+ 1.75	+ 2.54
legume	*Trifolium repens*	procedural	+ 1.47	+ 2.79
legume	*Medicago lupulina*	drought	+ 1.21	+ 3.47
grass	*Brachypodium pinnatum*	irrigated	+ 1.08	+ 1.69
grass	*Arrhenatherum elatius*	ambient	+ 0.92	+ 1.55

Table 2.13 Highest net proportionate increases in species abundance, 2016-2018.

plant group	species	rainfall treatment	area difference (m^2)	proportion difference
legume	*Lotus corniculatus*	drought	+ 0.83	17.60
forb	*Knautia arvensis*	procedural	+ 0.19	10.50
legume	*Trifolium repens*	irrigated	+ 4.28	8.25
grass	*Brachypodium pinnatum*	drought	+ 1.82	7.07
forb	*Taraxacum agg.*	procedural	+ 0.06	7.00
legume	*Medicago lupulina*	irrigated	+ 1.95	6.57
grass	*Agrostis capillaris*	ambient	+ 0.05	6.00
forb	*Cirsium eriophorum*	procedural	+ 0.19	5.75
forb	*Knautia arvensis*	irrigated	+ 0.09	5.50
forb	*Senecio erucifolius*	drought	+ 0.12	5.00

Trifolium repens, *Lotus corniculatus* and *Medicago lupulina* all had a net increase in abundance between 2016 and 2018. The single greatest gain was in *Trifolium repens*, which saw the highest area increase (4.28 m^2) and the highest proportionate increase (825%) across irrigated plots. *Lotus corniculatus* had the greatest proportionate increase (1760%) in drought plots, and the second highest area increase (2.35 m^2) in procedural control plots. *Brachypodium pinnatum*

(1.82 m^2 in drought plots, 1.08 m^2 in irrigated plots) and *Arrhenatherum elatius* (1.75 m^2 in procedural control plots and 0.92 m^2 in ambient controls) were the most expansive grasses in terms of area; *Agrostis capillaris* also made high proportionate increases (600%) in ambient control plots. Year-on-year changes were highly varied, e.g. *Brachypodium pinnatum* increased in abundance in all rainfall treatments between 2016 and 2017, then declined again in all but the drought plots between June 2017 and June 2018.

Trisetum flavescens declined across all rainfall treatments, as did *Clinopodium vulgare*; *Festuca rubra* decreased area cover in all but the ambient control plots in 2018 compared with 2016. The highest proportionate decreases in abundance were forb species; these species were predominantly of initially low cover, so the loss of one or a few plants is reflected as a large proportionate reduction in cover (Table 2.14, Table 2.15). *Hypericum perforatum* had retracted by over 90% in the irrigated plots, and *Potentilla repens* by a similar amount from ambient controls; in real terms, this equated to a contraction of 0.01 m^2 and 0.03 m^2 respectively. The gain in *Taraxacum* cover in procedural control plots in 2018 was offset by a loss of 89% of cover in ambient control plots.

Table 2.14 Highest net cover reduction in species abundance, 2016-2018.

plant group	species	rainfall treatment	area difference (m^2)	proportion difference
grass	*Trisetum flavescens*	drought	- 4.87	0.12
grass	*Trisetum flavescens*	ambient	- 4.72	0.16
grass	*Trisetum flavescens*	procedural	- 4.72	0.14
grass	*Trisetum flavescens*	irrigated	- 3.58	0.20
forb	*Clinopodium vulgare*	ambient	- 1.77	0.21
forb	*Clinopodium vulgare*	drought	- 1.67	0.41
forb	*Clinopodium vulgare*	procedural	- 1.59	0.31
grass	*Poa trivialis*	drought	- 1.29	0.05
forb	*Clinopodium vulgare*	irrigated	- 1.15	0.24
grass	*Festuca rubra*	drought	- 1.13	0.11

Table 2.15 Highest net proportionate reductions in species abundance, 2016-2018.

plant group	species	rainfall treatment	area difference (m^2)	proportion difference
forb	*Hypericum perforatum*	irrigated	- 0.01	0.94
forb	*Potentilla erecta*	ambient	- 0.03	0.93
forb	*Taraxacum agg.*	ambient	- 0.01	0.89
legume	*Vicia sativa*	irrigated	- 0.03	0.86
forb	*Geranium columbinum*	drought	- 0.03	0.86
grass	*Brachypodium sylvaticum*	drought	- 0.05	0.85
forb	*Crepis capillaris*	ambient	- 0.03	0.84
forb	*Glechoma hederacea*	ambient	- 0.01	0.83
forb	*Viola hirta*	ambient	- 0.01	0.83
grass	*Holcus lanatus*	procedural	- 0.10	0.81

2.4.4.2 Plant group abundance

Plant group abundance was sensitive to variations in soil moisture but showed no significant response to nitrogen treatment per se; when all three years' data were pooled, all significant interactions detected between rainfall and nitrogen treatments involved the nitrogen water-only control, further supporting the finding that moisture availability is the main driver behind many responses in this community. Responses in individual years (2017, 2018) showed a low level of sensitivity to nitrogen-addition treatments, though again this was dominated by the water control where a nitrogen response was significant.

In the baseline survey in June 2016, the sward was dominated by graminoid species, which accounted for a significantly higher proportion of total cover across all plots than all other plant groups ($t_{(553)} = 41.76$, $p < 2e\text{-}16$ ***) (Table 2.16). By June 2018, the sward was dominated by legume species, which showed a gradual increase in abundance year-on-year.

Table 2.16 Mean proportions of total quadrat plant cover of grass, forb, legume and woody species, 2016-2018, ± SD.

	plant group			
year	**grass**	**forbs**	**legumes**	**woody**
2016	0.563 ± 0.131	0.284 ± 0.109	0.143 ± 0.129	0.008 ± 0.011
2017	0.342 ± 0.132	0.367 ± 0.175	0.271 ± 0.196	0.019 ± 0.027
2018	0.318 ± 0.103	0.274 ± 0.136	0.401 ± 0.157	0.007 ± 0.012

Linear mixed effects models for graminoid abundance indicate that date accounted for greater variance (37% of total variance) than block (6% of total variance) across all three surveyed years, i.e. temporal variation through time was greater than spatial variation due to habitat heterogeneity. Baseline abundance levels for the three main cover classes (grass, forb, legume) were significant predictors of levels in subsequent years.

2.4.4.2.1 Grass

There was a site-wide reduction in grass cover in both 2017 and 2018 compared to the baseline survey in June 2016 (Figure 2.23). Once variation due to spatial heterogeneity had been accounted for, linear mixed effect models detected no significant differences between rainfall or nitrogen treatments in 2018, though grass cover was measurably lower in the drought plots compared with the other rainfall treatments (Table 2.17).

Table 2.17 Mean grass abundance by rainfall treatment. Grass abundance under drought treatment (2017) was the only significant response across both experimental years.

response	rainfall treatment	mean	SD	minimum	maximum
grass2017	ambient	0.349	0.163	0.12	0.70
	irrigated	0.393	0.222	0.09	0.93
	drought *	0.233	0.160	0.05	0.64
	procedural	0.243	0.100	0.08	0.43
grass2018	ambient	0.377	0.109	0.17	0.61
	irrigated	0.326	0.103	0.13	0.53
	drought	0.249	0.084	0.13	0.43
	procedural	0.281	0.111	0.12	0.45

The imposed drought treatment compounded existing water-stress caused by an early summer drought that year; this resulted in a significantly reduced grass abundance in the droughted

plots ($t_{(59.24)}$ = -2.556, p = 0.0132). Significant response variables from the models are given in Table 2.18 Significant fixed-effect responses from linear mixed effects models for grass abundance, where F1 = rainfall treatment, F2 = nitrogen treatment, covariate = baseline (2016) data, random = block.

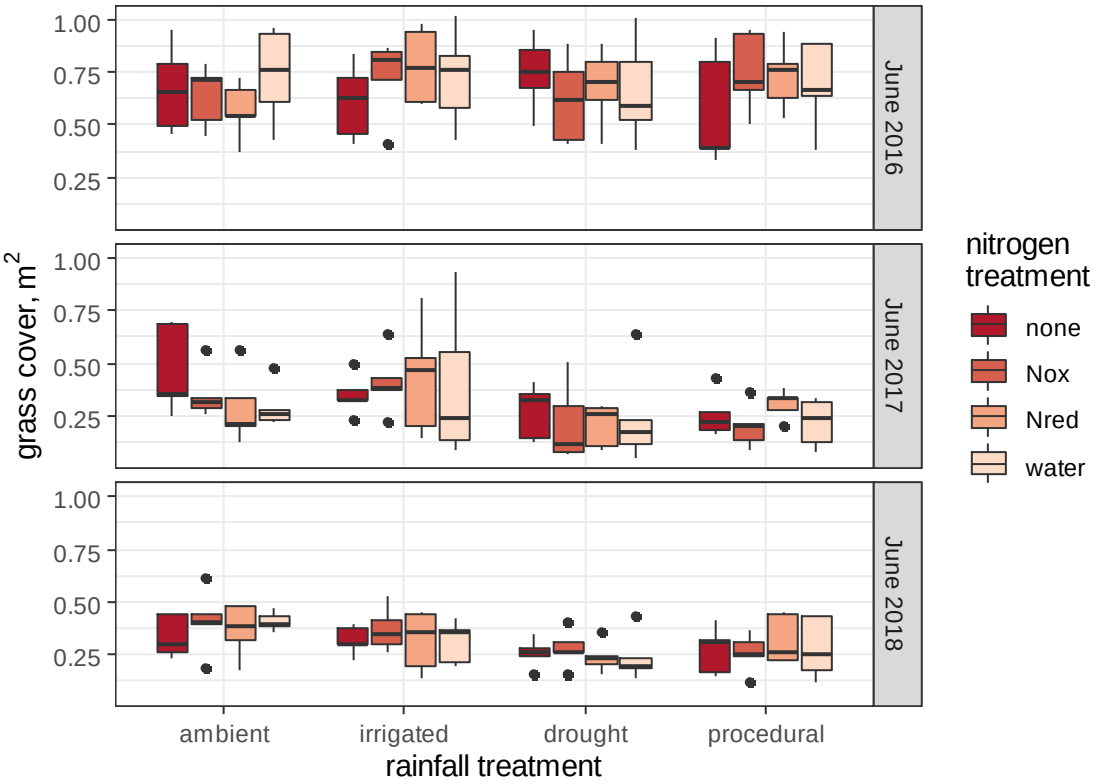


Figure 2.23 Grass abundance cover across all treatment combinations, 2016-2018.

*Table 2.18 Significant fixed-effect responses from linear mixed effects models for grass abundance, where F1 = rainfall treatment, F2 = nitrogen treatment, covariate = baseline (2016) data, random = block. Main effects are given as single treatment responses R_ (rainfall) or N_ (nitrogen); interaction responses are given in the form R_rainfall:N_nitrogen treatments. t-values are given to 3 decimal places; p-values are given 4 decimal places; degrees of freedom (df) are partial, and given to 2 decimal places; all other values are given to 4 decimal places. Significance levels: * p<0.05, ** p<0.01, *** p<0.001.*

model						
response	**estimate**	**SE**	**df**	**t-value**	**p**	
grass cover 2017 ~ F1 + F2 + F1:F2 + covariate + random						
grass cover 2016	0.4066	0.1039	62.31	3.915	0.0002	***
R_drought	-0.2289	0.0895	59.24	-2.556	0.0132	*
N_water	-0.1997	0.0895	59.22	-2.231	0.0295	*
grass cover 2018 ~ F1 + F2 + F1:F2 + covariate + random						
grass cover 2016	0.2080	0.0636	21.58	3.271	0.0036	**

2.4.4.2.2 Forb

Forb abundance was reduced across most quadrats compared to the baseline abundance recorded in 2016. Forb species cover showed no significant response to any rainfall or nitrogen treatments or their interactions in either of the two experimental years (Figure 2.24), though there were measurable differences between the treatments, with forb abundance being greatest in drought plots (Table 2.19). Baseline levels of forb abundance were significant predictors of forb abundance in both 2017 and 2018 (Table 2.20).

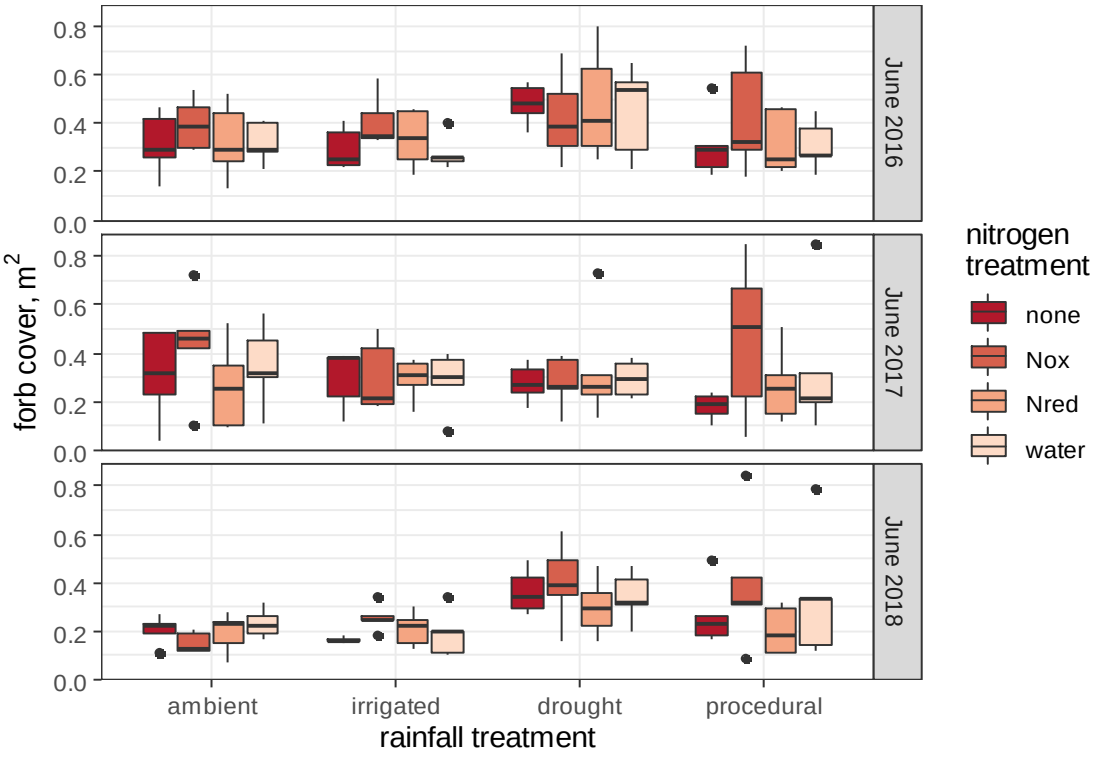


Figure 2.24 Forb abundance cover across all treatment combinations, 2016-2018.

Table 2.19 Mean forb abundance by rainfall treatment.

response	rainfall treatment	mean	SD	minimum	maximum
forb2017	ambient	0.340	0.187	0.04	0.72
	irrigated	0.294	0.113	0.08	0.50
	drought	0.295	0.130	0.12	0.73
	procedural	0.311	0.240	0.05	0.85
forb2018	ambient	0.196	0.065	0.07	0.32
	irrigated	0.205	0.070	0.10	0.34
	drought	0.351	0.120	0.16	0.61
	procedural	0.302	0.207	0.09	0.84

Table 2.20 Significant fixed-effect responses from linear mixed effects models for forb abundance. Details as per Table 2.18.

model						
response	**estimate**	**SE**	**df**	**t-value**	**p**	
forb cover 2017 ~ F1 + F2 + F1:F2 + covariate + random						
forb cover 2016	1.8272	0.4205	60.62	4.345	$5.39e^{-05}$	***
forb cover 2018 ~ F1 + F2 + F1:F2 + covariate + random						
forb cover 2016	1.410	0.3471	62.91	4.063	$1.37e^{-04}$	***

2.4.4.2.3 Legume

Legume cover increased over time, and was higher in 2018 across all treatment combinations compared to the baseline abundance levels (Figure 2.25).

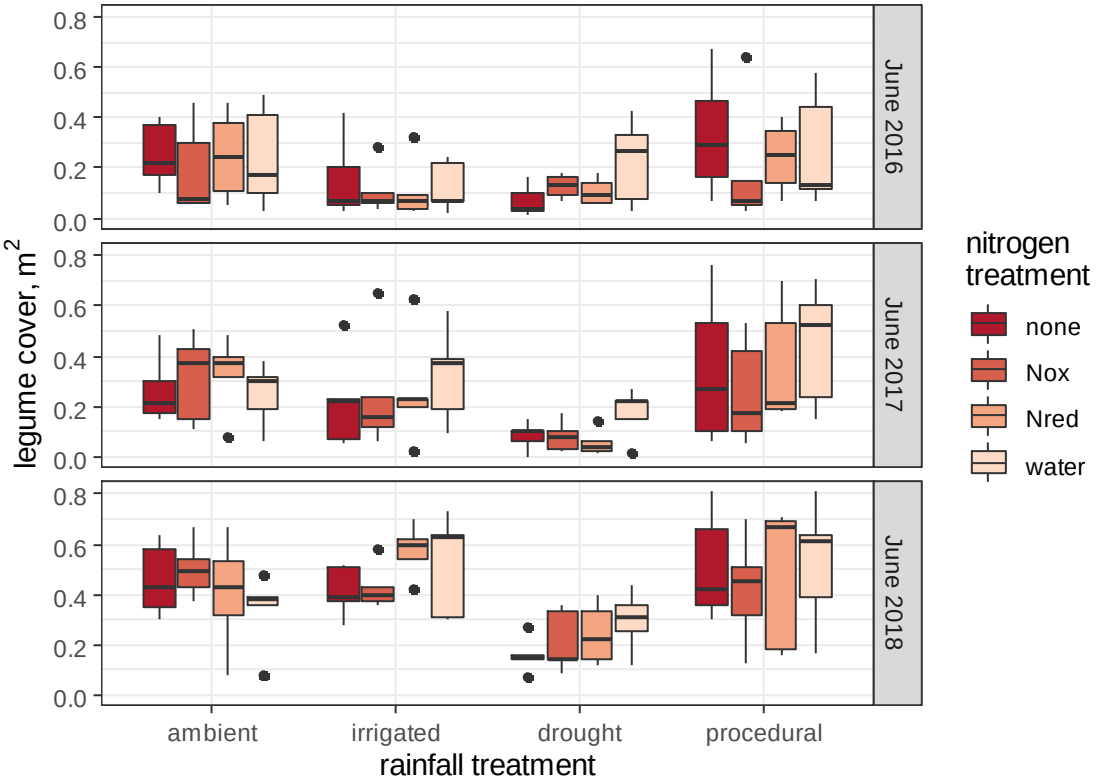


Figure 2.25 Legume abundance cover across all treatment combinations, 2016-2018.

Within-treatment changes in legume abundance appeared to be driven by changes in available moisture: by June 2018, legume cover in drought plots was significantly lower than ambient control plots (mean drought 0.227 m^2, mean ambient 0.426 m^2; $t_{(59.82)} = -2.404$, $p = 0.0193$). There was also significantly higher legume cover in plots in two of the irrigated:nitrogen

treatments (R_irrigated:N_Nred, $t_{(58.80)}$ = 2.199, p = 0.0313; R_irrigated:N_water, $t_{(58.78)}$ = 2.207. p = 0.0312) (Table 2.21).

Table 2.21 Significant fixed-effect responses from linear mixed effects models for legume abundance. Details as per Table 2.18.

model						
response	**estimate**	**SE**	**df**	**t-value**	**p**	
legume cover 2017 ~ F1 + F2 + F1:F2 + covariate + random						
legume cover 2016	0.829	0.1083	47.76	7.654	$7.61e^{-10}$	***
legume cover 2018 ~ F1 + F2 + F1:F2 + covariate + random						
legume cover 2016	0.600	0.1090	57.03	5.503	9.21e-07	***
R_drought	-0.192	0.0797	59.82	-2.404	0.0193	*
R_irrigated:N_Nred	0.240	0.1092	58.80	2.199	0.0313	*
R_irrigated:N_water	0.241	0.1091	58.78	2.207	0.0312	*

2.4.4.2.4 Relative plant group abundance and grass:forb ratio

Associated hypothesis:

H2.2: Grass to forb abundance ratio will increase under enhanced rainfall treatment compared to the drought treatment.

Grass cover declined between 2016 and 2018; legume species expanded proportionately as grass cover declined, in all but drought plots (Figure 2.26). Increased legume abundance took the form of both increased cover at existing (2016) locii, and also expansion into new quadrats.

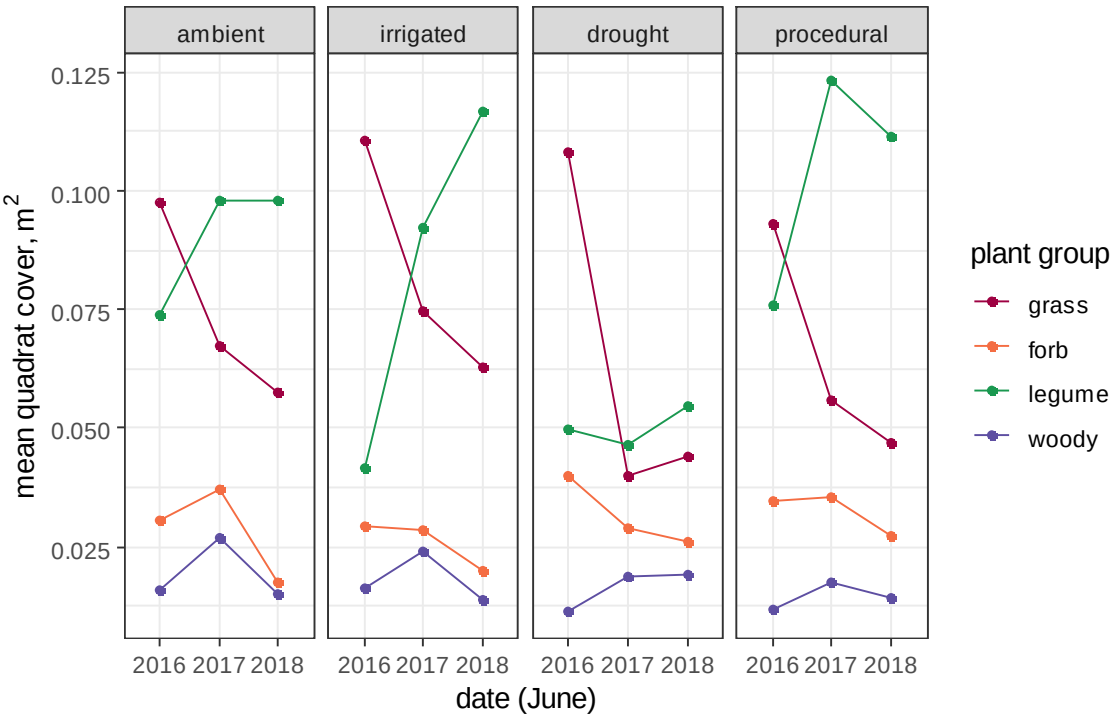


Figure 2.26 Mean quadrat cover by plant group, 2016-2018.

Changes in grass abundance between 2017 and 2018 were smaller and not significant, and grass abundance remained relatively low compared to the baseline. Legume cover continued to increase in irrigated and ambient control plots between 2017 and 2018, though not significantly; there was measurable but not significant change in legume cover in drought and procedural control plots, and though the magnitude of that change was comparable in the two treatments, they were in opposite directions.

Relative proportions of grass, forb and legume abundance illustrate changes in functional community composition. From a baseline position of dominance by grasses, there was a site-wide increase in legume abundance across all plots, with concurrent decrease in grass and forb abundance in 2018 compared with the baseline position (Figure 2.27).

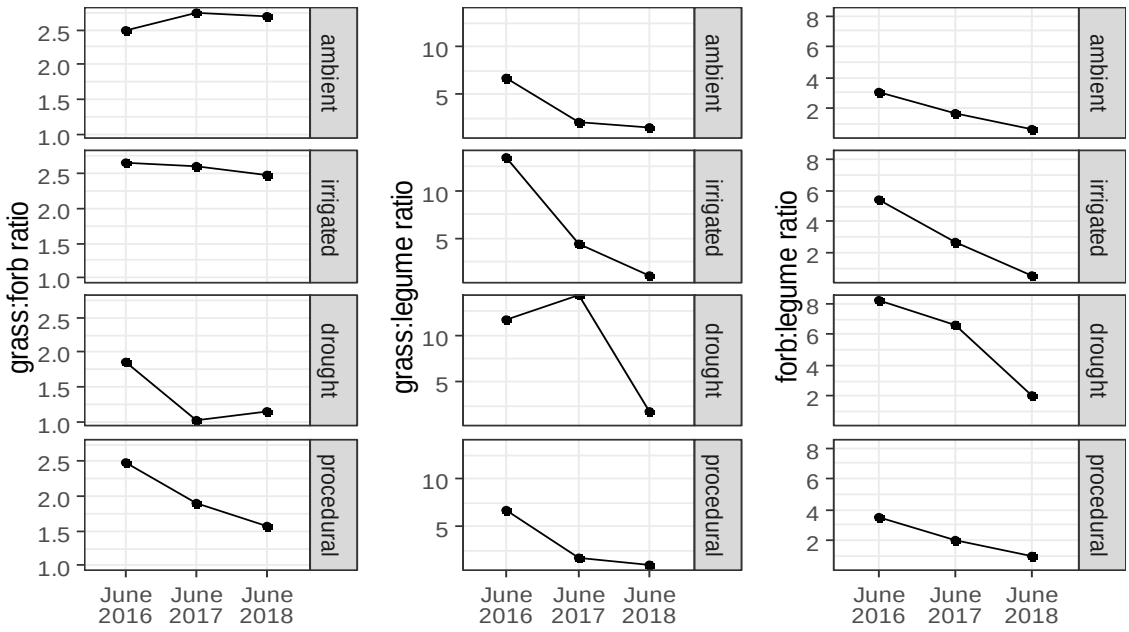


Figure 2.27 Mean plant group cover ratios at midseason surveys (June 2016 – June 2018).

Grass:forb ratio was calculated for all quadrats to investigate the effect of the nitrogen-addition treatments (Figure 2.29). Linear mixed effects models of grass:forbs ratio with rainfall and nitrogen treatments as main and interactive effects for each survey indicated that grass:forb ratio in the drought treatment plots were lower than the ratio in the other rainfall treatments; this was significant for 2018 grass:forb data ($t_{(60)}$ = -2.731, p = 0.00828) (Table 2.22).

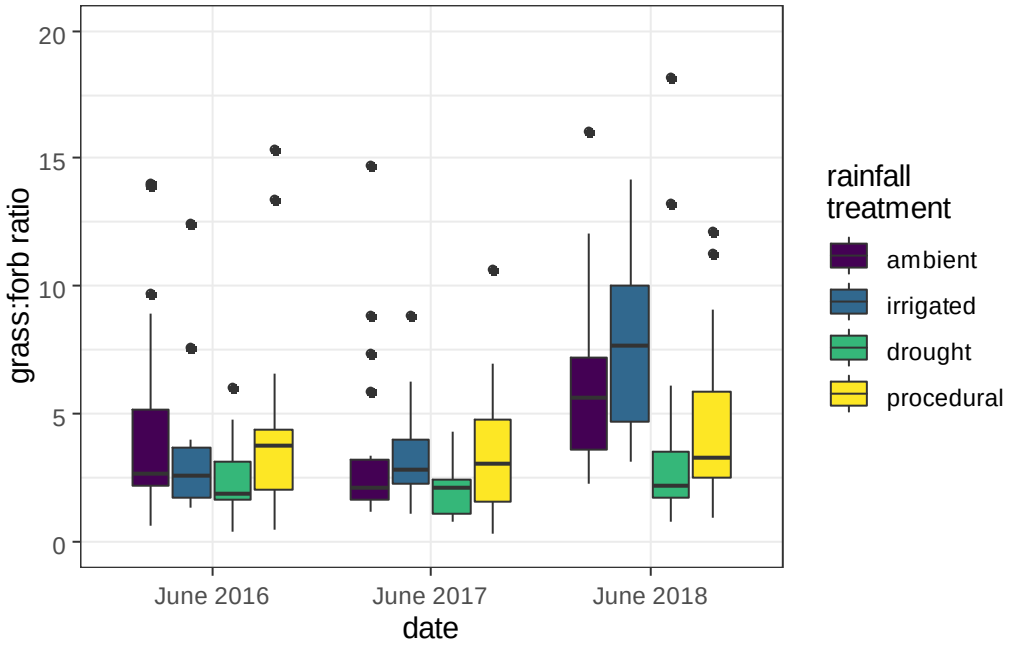


Figure 2.28 Grass:forb abundance ratio, across all rainfall treatments.

Table 2.22 Significant fixed-effect responses from linear mixed effects models for grass:forb ratio in 2018. Details as per Table 2.18.

model						
response	**estimate**	**SE**	**df**	**t-value**	**p**	
grass:forb ratio 2017 ~ F1 + F2 + F1:F2 + C1 + random						
Significant pairwise comparisons for total biomass, 2017						
comparison	estimate	SE	df	t-ratio	p	
drought – irrigated	0.4448	0.164	59.0	2.708	0.0427	*
grass:forb ratio 2018 ~ F1 + F2 + F1:F2 + C1 + random						
R_drought	-0.863	0.316	60.00	-2.731	0.00828	**
Significant pairwise comparisons for total biomass, 2018						
comparison	estimate	SE	df	t-ratio	p	
ambient – drought	0.433	0.155	60.3	2.793	0.0343	
irrigated – procedural	0.549	0.149	59.1	3.683	0.0027	
irrigated – drought	0,694	0.149	59.1	4.664	0.0001	

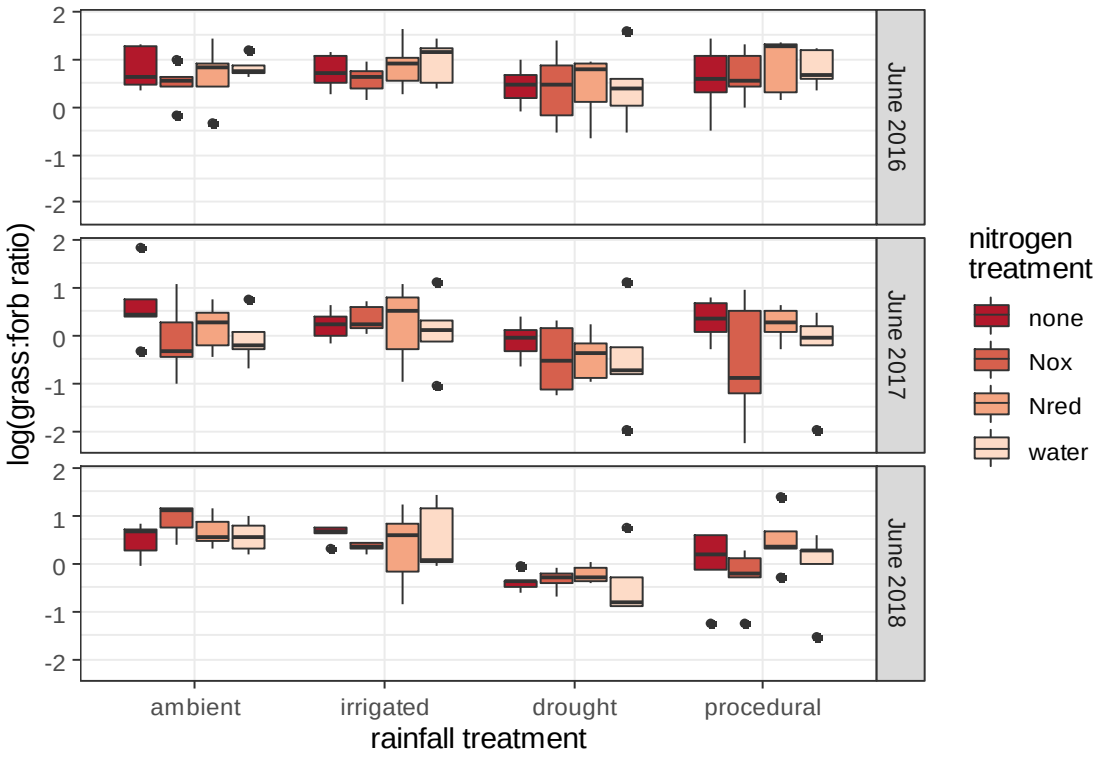


Figure 2.29 Grass:forb ratio across all treatment combinations.

Interactions between rainfall and nitrogen treatments were assessed for influence on grass:forb ratio. The interaction of the water control with the irrigated treatment in 2017 was associated with a higher grass:forb ratio compared to the previous year, suggesting a shift in plant group

abundance driven by the low rainfall over winter 2016/spring 2017. Water-only control quadrats returned the highest grass:forb ratio of the four nitrogen-addition treatments in the irrigated and drought plots; and the lowest ratio in the ambient and procedural control plots in 2017 (Figure 2.30, Table 2.23).

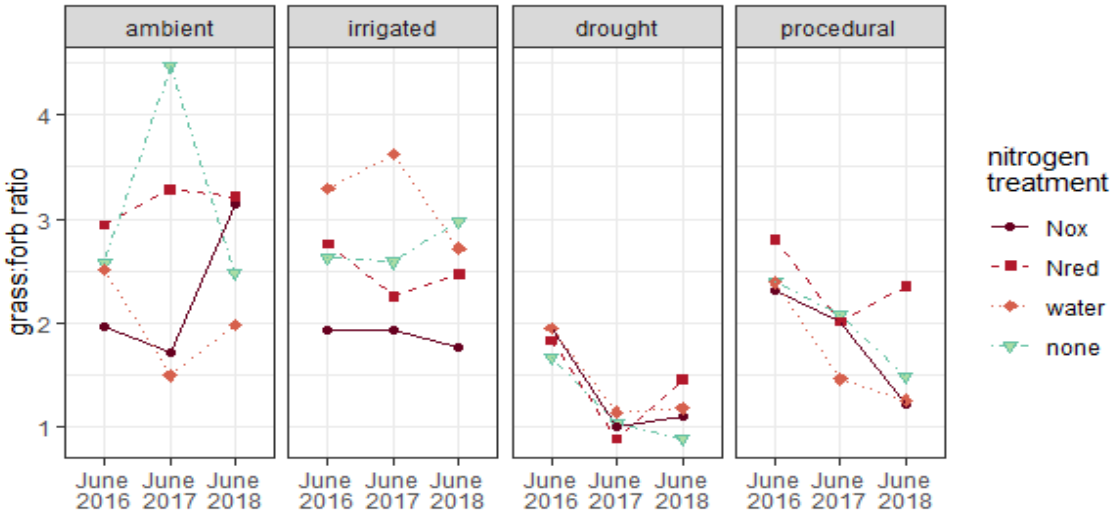


Figure 2.30 Mean grass:forb ratio - response to nitrogen and rainfall treatments.

Table 2.23 Mean grass:forb ratio for each rainfall/nitrogen treatment combination. For each case, n = 5.

		rainfall treatment			
year	**nitrogen treatment**	**ambient**	**irrigated**	**drought**	**procedural**
2017	Nox	1.72	1.93	1.01	2.02
	Nred	3.28	2.26	0.89	2.01
	water	1.50	3.62	1.14	1.46
	none	4.46	2.59	1.04	2.07
2018	Nox	3.13	1.77	1.10	1.22
	Nred	3.21	2.47	1.46	2.35
	water	1.98	2.72	1.18	1.26
	none	2.48	2.97	0.89	1.48

It was hypothesised (H2.2) that the grass:forb abundance ratio would increase in the nitrogen-addition plots under enhanced rainfall treatment compared to the drought treatment. Both Figure 2.29 and Table 2.23 indicate that the grass:forb ratio was higher under the irrigated treatment than under the drought treatment, across all nitrogen treatments, so Hypothesis 2.2 was supported by the data.

This was further investigated by normalising grass:forb ratios around the no-additions nitrogen

control (“none”) (Figure 2.31). The water addition response has been included in both the plot and the table as an additional comparison, as this was a second procedural control for the water used to deliver the aqueous nitrogen (Table 2.24). Due to the variation seen in the response in the two years, it is pertinent to bear in mind that 2017 saw a low rainfall in Spring and early Summer, leading to an early-season drought, whereas this was not the case for 2018.

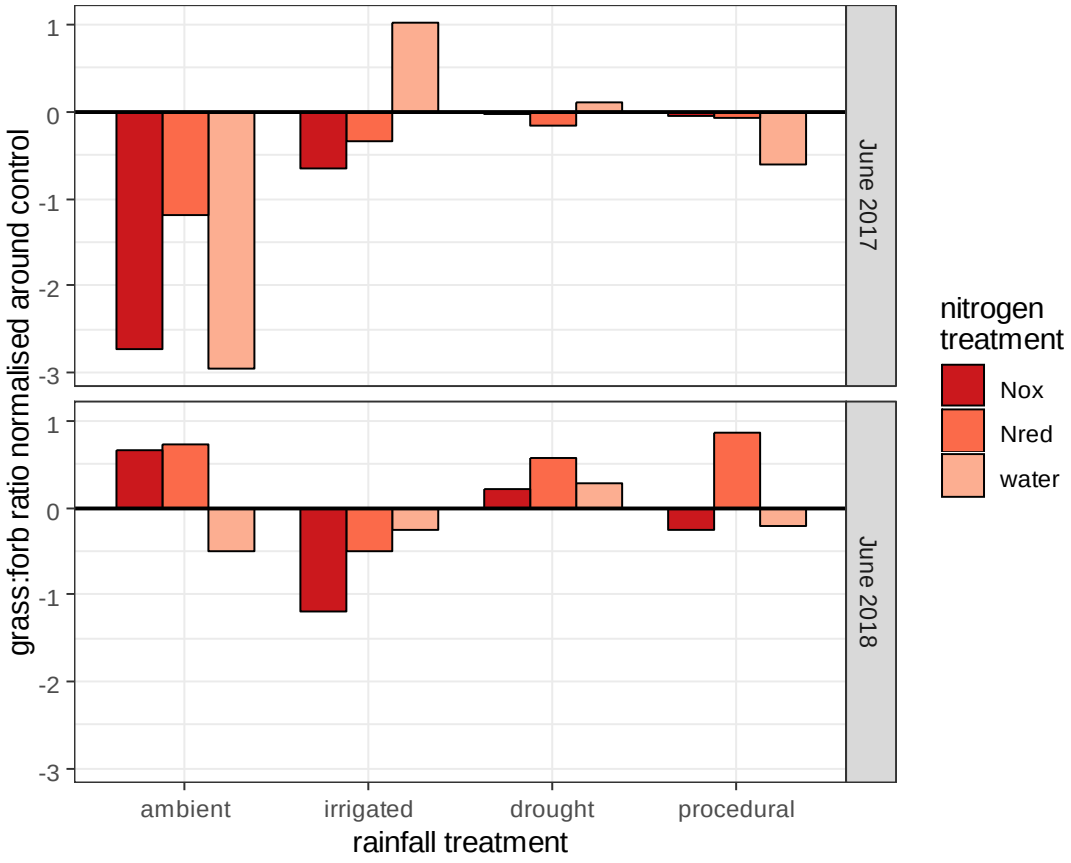


Figure 2.31 Grass:forb abundance ratio normalised around the "none" nitrogen treatment, being the no-additions control.

Results showed that grass:forb ratios under both oxidised and reduced nitrogen additions were less than in the no-treatment control quadrats in the drought and irrigated plots in 2017, and that the differential was higher with the addition of oxidised nitrogen in the irrigated plots than in the drought plots for both forms of nitrogen. This was contrary to the grass:forb response in the water-only control quadrats, which returned higher ratios in the two nitrogen addition treatments compared to the control; although the differential was again greater in the irrigated plots than in the drought plots; in this case it was positive. The greatest overall response was from the ambient control plots, for all three addition treatments (Nox, Nred and water), each of which has greatly reduced grass:forbs ratios in 2017, compared with the no-addition control. This was linked to a relative decrease in overall grass cover in ambient plots in 2017, when forb cover remained very close to the 2016 level.

The picture presented by grass:forb ratios the following year was different in magnitude and direction of responses. Grass:forb ratio in drought and ambient control plots were greater than in the control, whereas irrigated and procedural control plots had proportionately less grass abundance. Only in the procedural control plots did grass:forb ratios respond in opposite directions, with forb abundance apparently advantaged by oxidised nitrogen, and reduced nitrogen benefitting grass abundance. A clear difference could be seen in responses of the drought and irrigated plots; nitrogen-addition and the water control plots all favoured forb abundance in the irrigated plots, where the converse was true of the drought plots, i.e. there was proportionately more grass cover in drought plots compared to both the nitrogen control, and the irrigated plots as a group.

Table 2.24 Normalised values for grass:forb ratios, across all rainfall - nitrogen treatment combinations for the two years post-baseline.

		rainfall treatment			
year	**nitrogen treatment**	**ambient**	**irrigated**	**drought**	**procedural**
2017	Nox	-2.74	-0.66	-0.03	-0.05
	Nred	-1.18	-0.33	-0.15	-0.06
	water	-2.96	1.03	0.10	-0.61
2018	Nox	0.65	-1.2	0.21	-0.26
	Nred	0.73	-0.50	0.57	0.87
	water	-0.50	-0.25	0.29	-0.22

2.4.4.3 Life history abundance

Perennial species accounted for the majority of plant cover in all three survey years (90% in June 2016, 92% in June 2017, and 93% in June 2018). The vegetation on site was dominated by perennial grasses at commencement of the experiment in 2016 (53% of total cover in survey quadrats); perennial forbs accounted for 22% of total cover, and perennial legumes for 13% of total cover. All biennial cover on site was from forb species. Annual species' abundance declined year on year, across all plant groups and rainfall treatments. The number of annual species observed across all the survey quadrats remained fairly stable (13 species in 2016, 11 in 2017, 16 in 2018), though individual species presence/absence varied through the three surveyed years as conditions within the treatment plots became more or less amenable to them.

2.4.4.3.1 Grass

Perennial and annual grass cover diminished across the site in both 2017 and 2018 compared with the 2016 baseline cover abundance (Figure 2.32). Linear mixed effects models failed to detect a significant difference in the perennial grass abundance response to either rainfall or nitrogen treatments or their interaction in both 2017 and 2018, though the effect of rainfall treatment was significant when nitrogen-treatment effects were held at zero (Table 2.25). For both 2017 and 2018, drought plots had the lowest abundance cover (Table 2.26). No significant responses to nitrogen treatment were detected in perennial grass cover in either 2017 or 2018.

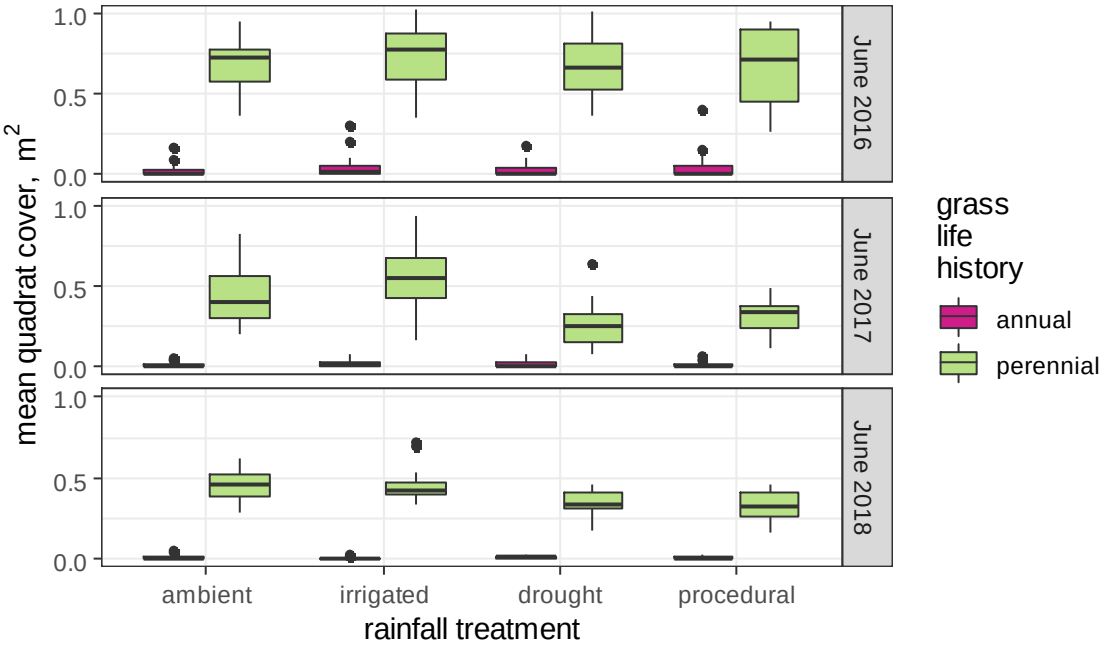


Figure 2.32 Grass abundance by life history.

Table 2.25 Significant responses from linear mixed effects model of perennial grass cover in 2017 and 2018, when nitrogen effects are held at zero. Details as per Table 2.18.

model						
response	**estimate**	**SE**	**df**	**t-value**	**p**	
perennial grass17 ~ F1 + covariate + random						
R_procedural	-0.1121	0.0416	71.10	-2.694	0.00881	**
R_drought	-0.1338	0.0417	71.12	-3.213	0.00198	**
perennial grass18 ~ F1 + covariate + random						
R_procedural	-0.0931	0.0293	75.00	-3.179	0.0022	**
R_drought	-0.1330	0.0293	75.00	-4.539	$2.12e^{-05}$	***

Table 2.26 Mean grass cover (m^2) by life history across rainfall treatments, 2017 and 2018.

		perennial grass		annual grass	
date	**rainfall treatment**	**mean**	**SD**	**mean**	**SD**
2017	ambient	0.3395	0.1652	0.0090	0.0159
	irrigated	0.3735	0.2228	0.0195	0.0206
	drought	0.2325	0.1001	0.0100	0.0159
	procedural	0.2165	0.1548	0.0160	0.0214
2018	ambient	0.3670	0.1052	0.0100	0.0156
	irrigated	0.3230	0.1022	0.0025	0.0055
	drought	0.2765	0.1117	0.0040	0.0060
	procedural	0.2395	0.0867	0.0095	0.0076

Linear mixed effect models indicated a significant relationship between annual grass cover and the oxidised nitrogen treatment (Nox) in 2018, though not in 2017 (Table 2.27). The interactions between Nox and the procedural control and drought rainfall treatments were both negative and significant (Table 2.27).

Table 2.27 Significant responses from linear mixed effect model of annual grass cover abundance in 2018. Details as per Table 2.18.

model						
response	**estimate**	**SE**	**df**	**t-value**	**p**	
annual grass 18 ~ F1 + F2 + F1:F2 + covariate + random						
N_Nox	0.01444	0.0053	58.99	2.718	0.0086	**
R_procedural:N_Nox	-0.0206	0.0075	58.98	-2.752	0.0079	**
R_drought:N_Nox	-0.0170	0.0075	59.01	-2/255	0.0279	*

2.4.4.3.2 Forb

Linear mixed effect models found no significant responses in annual or biennial forb abundance to rainfall or nitrogen treatments. By 2018, perennial forb species were significantly more abundant under the experimental drought conditions; pairwise comparisons indicated that perennial forb abundance was significantly greater in drought compared with the ambient control plots (ambient control : drought control, t-ratio$_{(59.3)}$ = -3.239, p = 0.0103*) and also in drought plots compared with irrigated plots (irrigated : drought, t-ratio$_{(59.4)}$ = -2.694, p = 0.0442). Perennial forb cover was lowest in ambient control plots in 2018; in the two previous years,

irrigated plots had lowest perennial forb cover (Figure 2.33). Annual forb abundance was not significantly influenced by the imposed rainfall or nitrogen treatments.

Table 2.28 Significant abundance response by perennial forbs; comparison with 2016 baseline data.

model						
response	**estimate**	**SE**	**df**	**t-value**	**p**	
perennial forb 17 ~ F1 + F2 + F1:F2 + random						
procedural control	-0.0960	0.0474	60.00	-2.024	0.0474	*
perennial forb 18 ~ F1 + F2 + F1:F2 + random						
drought	0.0756	0.0347	60.00	2.176	0.0335	*

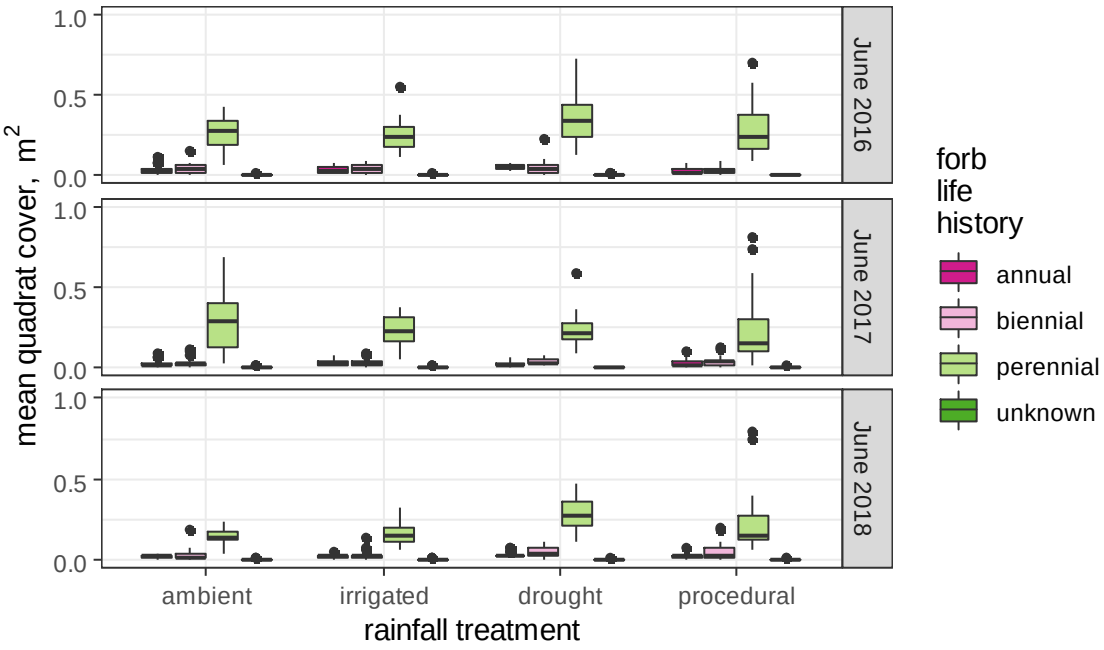


Figure 2.33 Forb abundance by life history.

2.4.4.3.3 Legume

Annual legume abundance showed a net small decrease between June 2016 and June 2018, with a larger (though not significant) decrease across all rainfall treatments in 2017. Perennial legume abundance increased year on year in all rainfall treatments and in both experimental years, apart from in the drought treatment in 2017, where the combination of imposed summer drought and lower Spring rainfall reduced perennial legume abundance to a mean quadrat abundance of 0.10 m^2 compared to 0.12 m^2 in the baseline survey in 2016, and 0.22 m^2 in June 2018 (Figure 2.34).

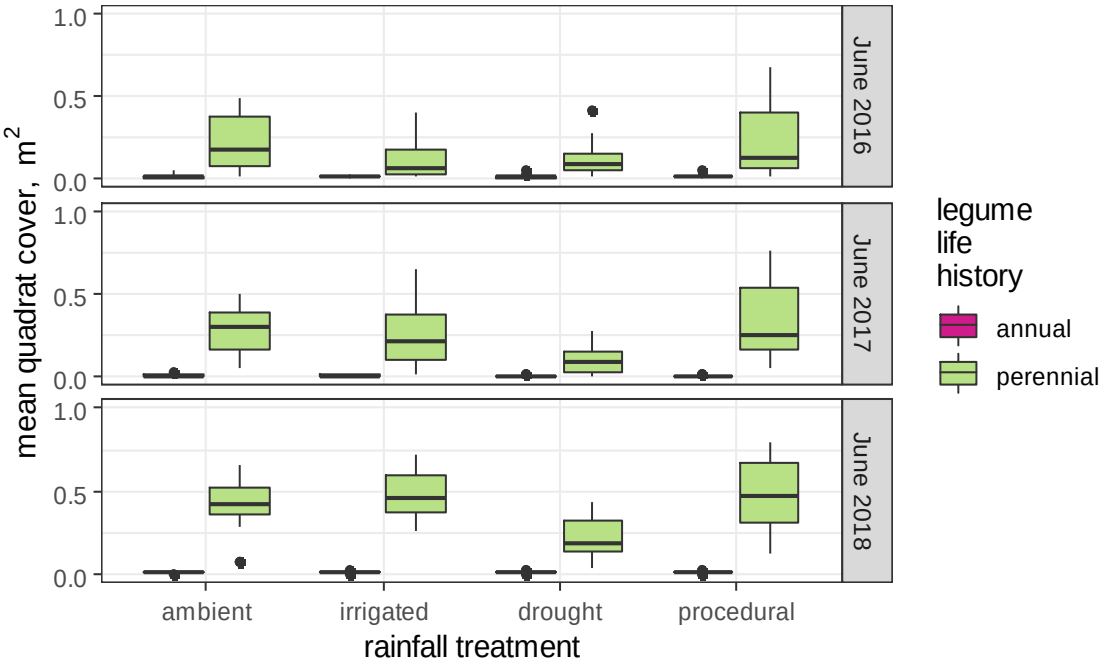


Figure 2.34 Legume abundance by life history.

Although perennial legume abundance did not respond significantly to the nitrogen treatments, there were multiple significant pairwise comparisons to rainfall treatment when least squares means were averaged across all levels of nitrogen treatment and the baseline abundances taken into account (Table 2.29).

Table 2.29 Pairwise comparisons for perennial legume cover ~ rainfall treatment, 2018. P-adjustment by Tukey method, using Kenward-Roger degrees-of-freedom method.

Pairwise comparisons for perennial legume cover, 2018					
comparison	estimate	SE	df	t-ratio	p
ambient : irrigated	-0.1219	0.0404	60.4	-3.018	0.0190
ambient : drought	0.1413	0.0401	60.2	3.521	0.0045
irrigated : drought	0.2632	0.0385	59.0	6.843	<-.0001
procedural : drought	0.1858	0.0410	60.7	4.537	0.0002

2.4.4.4 C-S-R class abundance

Rainfall manipulations were found to influence the relative abundance of C-S-R classes in the four treatments (Figure 2.35). Significant abundance responses by C-S-R classes are given in Table 2.30, below.

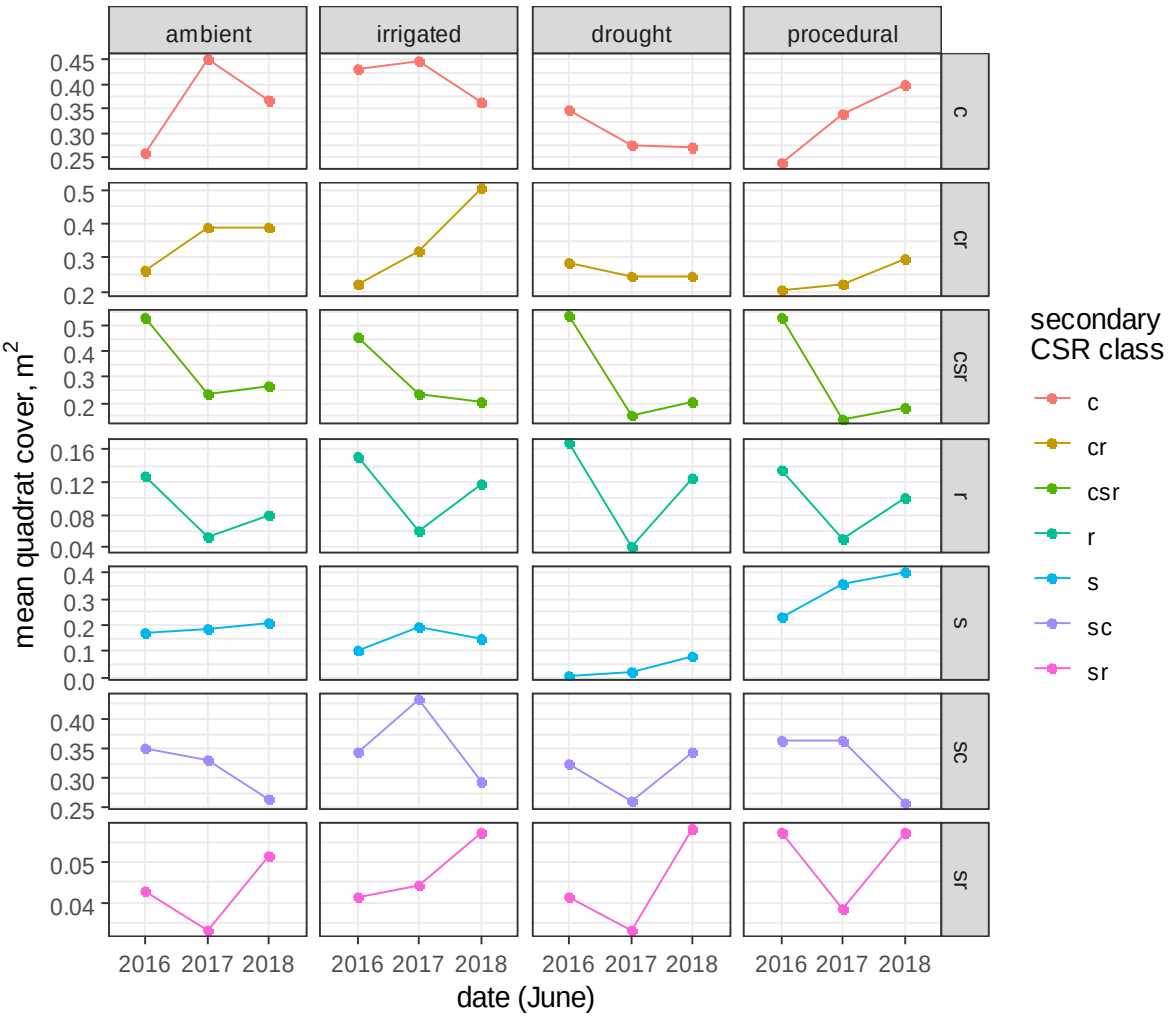


Figure 2.35 Mean cover of secondary C-S-R classes in quadrats, 2016-2018. C-S-R classes are abbreviated as follows: c = C competitive, cr = CR competitive-ruderal, csr = CSR generalist, r = R ruderal, s = S stress-tolerant, sc = SC stress-tolerant competitive, sr = SR stress-tolerant ruderal.

Table 2.30 Significant results of linear mixed effects model for C-S-R class abundance responses to rainfall and nitrogen treatments.

model						
response	**estimate**	**SE**	**df**	**t-value**	**p**	
C-S-R group abundance 2017 ~ F1 + F2 + F1:F2 + random						
generalist CSR	-0.2307	0.0643	444.00	-3.587	3.71e-04	***
ruderal R	-0.1464	0.0643	444.00	-2.277	0.0233	*
irrigated:Nred:CR	-0.2891	0.1286	444.00	-2.248	0.0251	*
procedural:Nwater:SC	0.3729	0.1286	444.00	2.900	0.00392	**
C-S-R group abundance 2018 ~ F1 + F2 + F1:F2 + random						
generalist CSR	-0.1292	0.0655	444.00	-1.974	0.0490	*
Nox:S	-0.1863	0.0926	444.00	-2.013	0.0448	*
drought:Nwater:CSR	0.2665	0.1309	444.00	2.036	0.0424	*
drought:Nwater:R	0.2989	0.1309	444.00	2.283	0.0229	*
procedural:Nwater:S	0.3074	0.1309	444.00	2.348	0.0193	*

Generalist (CSR) abundance declined across the board in the two years following the baseline survey, primarily due to large-scale reductions of *Trisetum flavescens* and *Poa trivialis* from all rainfall treatments; also *Festuca rubra* from drought and procedural control plots. Ruderal (R) abundance fell in all rainfall treatments in 2017 and remained lower thereafter.

Stress-tolerant species in C-S-R class S were significantly less abundant in oxidised nitrogen treatments (Nox) in June 2018 than they had been in 2016. There was a general trend for CSR, R and S-class plants to be more abundant in drought plots in June 2018 than in 2016; this increased abundance was significant for CSR and R abundance in the nitrogen water-control plots. Stress-tolerator abundance was significantly greater in the nitrogen water-control plots under the procedural control shelters than it had been in 2016.

Trends seen in C-S-R-class abundance over the two experiment years indicated a degree of variability in response to rainfall treatments – this was interpreted in part in the context of the relatively low Spring rainfall in 2017. Following the drier Spring of 2017, C, CR and CSR-class species were most abundant in ambient control plots; this association of C-S-R-class to particular rainfall treatment was less clear in 2018, when Spring rainfall had been higher. The

large overall increase in CR species abundance was driven mainly by an overall increase in *Trifolium repens* (white clover) in irrigated plots. Stress-tolerant species were most abundant in procedural control plots in both 2017 and 2018. R, SC and SR species were all most abundant in the irrigated plots in 2017, and the drought treatment plots in 2018.

With the exception of stress-tolerant species being most abundant in procedural control plots, all C-S-R classes had their highest mean cover in ambient or irrigated plots, i.e. those with higher soil moisture content (Table 2.31).

Table 2.31 Association of highest mean abundance and rainfall treatments, 2016-2018.

C-S-R class	2016	2017	2018
c	irrigated	ambient	procedural
cr	drought	ambient	irrigated
csr	drought	ambient	ambient
r	drought	irrigated	drought
s	procedural	procedural	procedural
sc	procedural	irrigated	drought
sr	procedural	irrigated	drought

Eighteen of the 19 annual species observed across the three midseason surveys were more ruderal or competitive-ruderal species (*sensu* Grime, 1977), for example *Vicia sativa*, which was present in survey quadrats in all three years, is a stress-tolerant ruderal species.

It was hypothesised (H2.3) that the competitive fraction of the community would increase under the irrigation treatment compared with that in the drought plots. This was tested on abundance data using linear mixed effect models, which supported the hypothesis for 2017 but not for 2018 (Table 2.32), though there was a clearly observable effect in 2018 (Figure 2.36). There were no significant effects on Grime C of the interaction between rainfall and nitrogen treatments in 2017 or 2018.

Table 2.32 Significant output for linear mixed effect models of Grime C abundance under rainfall and nitrogen treatments for 2017 and 2018.

***model*: Grime C ~ F1 + F2 + F1:F2 + C1 + random**					
response	**estimate**	**SE**	**df**	**t-value**	**p**
drought (2017)	-0.0018	0.0007	59.04	-2.583	0.012
drought (2018)	-0.0009	0.0006	59.14	-1.567	0.122

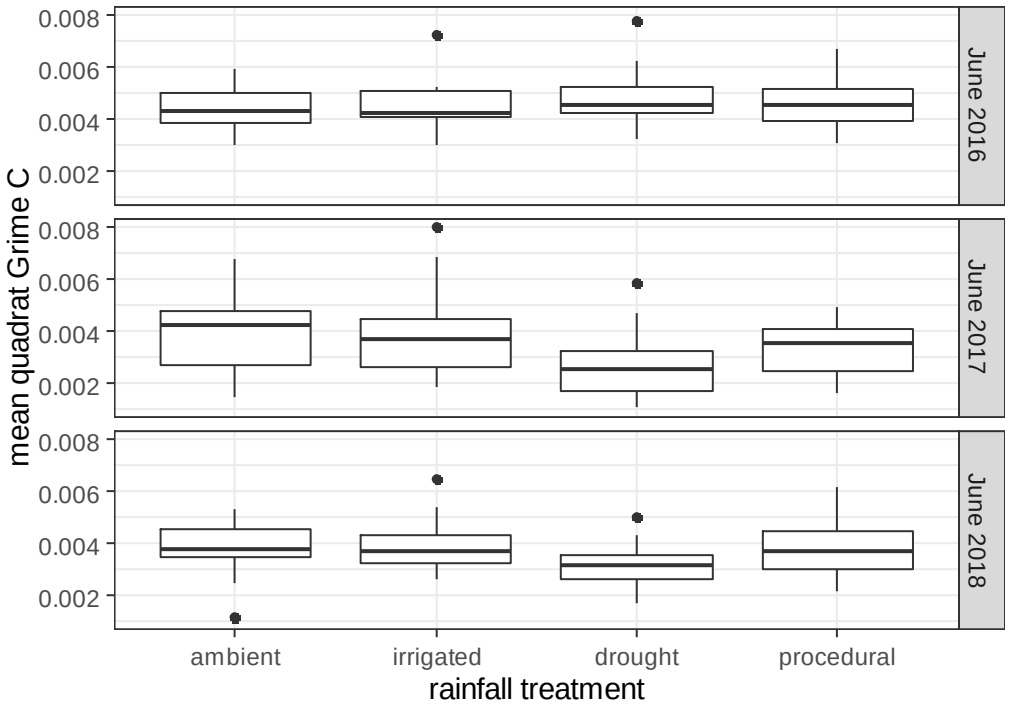


Figure 2.36 Influence of rainfall treatment on Grime C for 2016-2018.

Least-squares pairwise comparisons indicated that the Grime C fraction was significantly greater under irrigation than under drought, in both 2017 and 2018 (Table 2.33), when averaged over all levels of nitrogen treatment.

Table 2.33 Pairwise comparisons for perennial legume cover ~ rainfall treatment, 2018. P-adjustment by Tukey method, using Kenward-Roger degrees-of-freedom method.

Pairwise comparisons for Grime C under rainfall treatment					
comparison	estimate	SE	df	t-ratio	p
irrigated – drought 2017	0.0014	0.0004	59.2	3.819	0.0018
irrigated – drought 2018	0.0009	0.0003	59.2	3.170	0.0125

2.4.5 Biomass responses

Associated hypotheses:

- H2.4: Reduction in above-ground biomass under the drought treatment will be proportionally similar to an increase in above-ground biomass under the enhanced rainfall treatment.

- H2.5: Biomass will increase in response to nitrogen addition, where moisture availability is sufficient.

2.4.5.1 Baseline and variation between harvests

Species surveys and biomass harvests were made at midsummer and end of season (late June and late September) in 2016 and 2017, and midsummer surveys only in 2018. Total above-ground biomass for all five harvests appeared to be strongly associated with rainfall in the preceding three months, and this was particularly evident for the three midseason harvests (Figure 2.37); total biomass in both experimental years was much reduced compared to the 2016 baseline.

The site-wide reduction in productivity between June 2016 and June 2017 may have been due at least in part to the change in management from being sheep-grazed to a hay-cutting regime, whereby nutrients previously returned to and stored in the soil through dunging were subsequently lost to the system. The plant biomass supported by this (in early and mid-season growth in the first year post-management change) was lost to the site system following the hay cuts in June and September, as all arisings were removed from site. Also, spring and early summer 2016 were wet and warm, and productivity was acknowledged anecdotally by the estate manager to be uncharacteristically high.

Figure 2.37 Total harvested biomass plotted with mean monthly temperature and total seasonal precipitation.

End of season biomass harvest in 2016 was considerably less than the midseason biomass three months previously, contributing 8% of the total biomass over the growing season (680 g compared to 7820 g in summer). End of season biomass in 2017 contributed 30% (1588 g) of the total biomass over the growing season, reflecting the greater summer rainfall. Due to time constraints, end of season biomass was not sampled in 2018, and the following analysis refers to midseason biomass only.

2.4.5.2 Total quadrat biomass response to experimental treatments

Associated hypothesis:

- H2.5 Biomass will increase in response to nitrogen addition, where moisture availability is sufficient.

When averaged over all levels of nitrogen treatment, total biomass was significantly lower in drought treatment plots compared with the other three rainfall treatments in both 2017 and 2018 (Figure 2.38, Table 2.34).

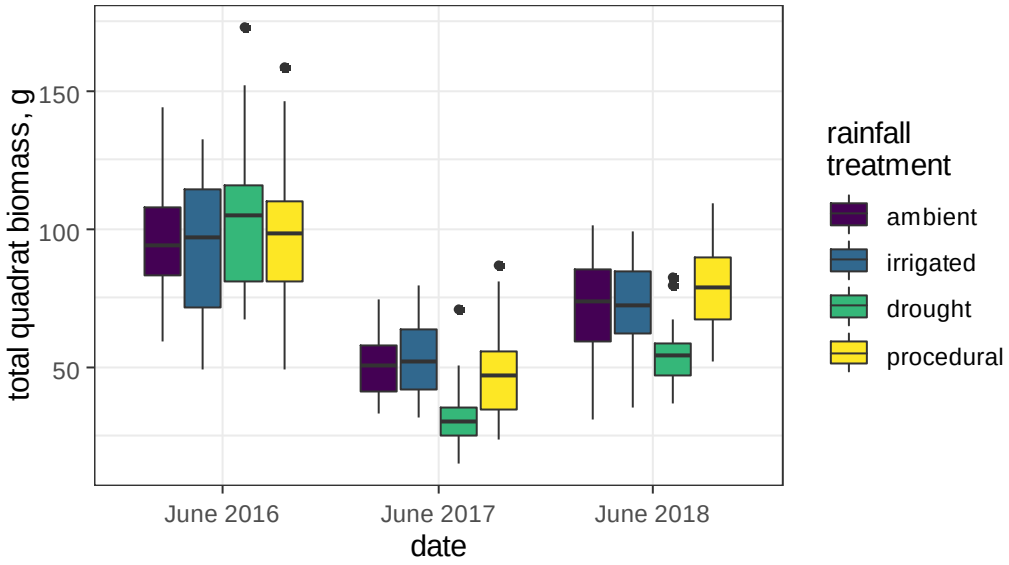


Figure 2.38 Mean total biomass response to rainfall treatment. June 2016 is the baseline harvest, taken prior to the experiment treatments being imposed.

There was no significant response of total biomass to nitrogen treatment when averaged over all levels of rainfall treatment in either 2017 or 2018 (Figure 2.39).

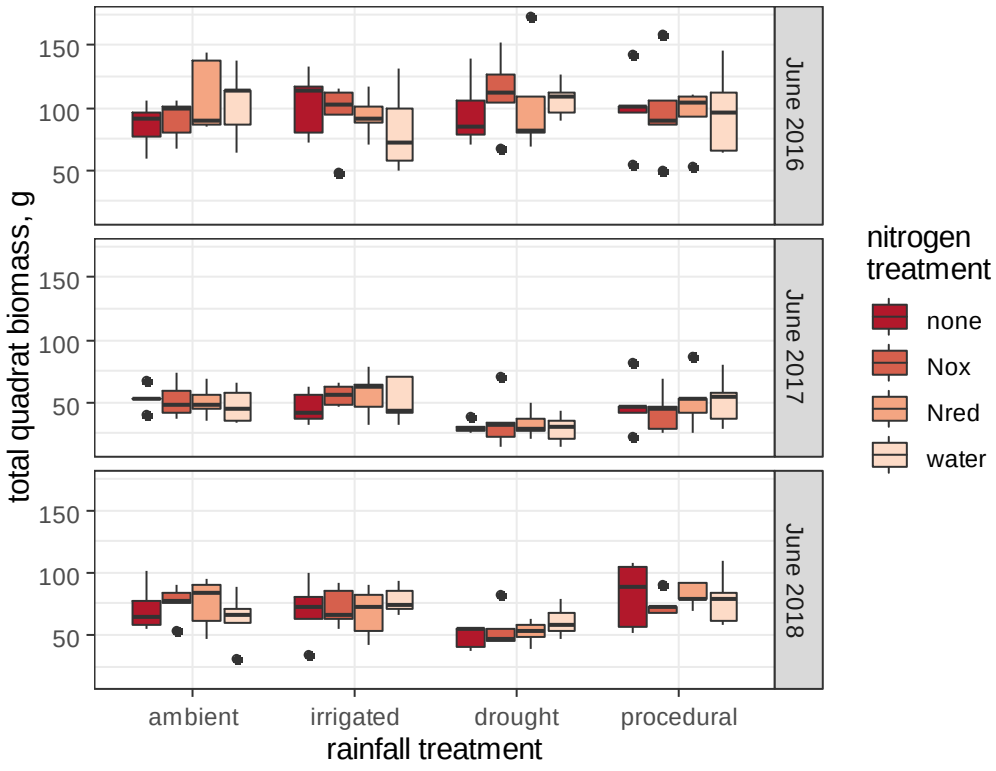


Figure 2.39 Total quadrat biomass responses to rainfall and nitrogen treatments.

Pairwise comparisons across all levels of both treatments indicated multiple significant differences in total quadrat biomass between some treatment combinations (Table 2.34); all the

significant treatment contrasts in 2017 involved the water-only control, and three of these were comparing with irrigated plots.

Table 2.34 Significant results of linear mixed effects model for total biomass response to rainfall and nitrogen treatments. df = Kenward-Roger partial estimation, p-value adjustment is the Tukey method.

***model*: total biomass 2017 ~ F1 + F2 + F1:F2 + C1 + random**					
response	**estimate**	**SE**	**df**	**t-value**	**p**
drought	-0.3942	0.1960	63.00	-3.032	0.0035 **
Significant pairwise comparisons for total biomass, 2017					
comparison	estimate	SE	df	t-ratio	p
ambient : drought	0.5371	0.0983	59.3	5.461	<0.0001
irrigated : drought	0.5845	0.0991	59.6	5.900	<0.0001
procedural : drought	0.4556	0.0984	59.3	4.631	0.0001
ambient_none:drought_water	0.7602	0.198	59.6	3.842	0.0237
irrigated_Nox:drought_water	0.7643	0.196	59.2	3.893	0.0205
irrigate_Nred:drought_water	0.7603	0.196	59.3	3.870	0.0219
irrigate_water:drought_water	0.7251	0.199	59.8	3.646	0.0413
***model:* total biomass 2018 ~ F1 + F2 + F1:F2 + C1 + random**					
response	**estimate**	**SE**	**df**	**t-value**	**p**
drought	-0.4010	0.1483	59.13	-2.704	0.0089 **
Significant pairwise comparisons for total biomass, 2018					
comparison	estimate	SE	df	t-ratio	p
ambient : drought	0.2823	0.0744	59.1	3.793	0.0019
irrigated : drought	0.2998	0.0750	59.3	3.998	0.0010
procedural : drought	0.3932	0.0744	59.2	5.281	<0.0001
drought_none:procedural_Nred	-0.5370	0.148	59.0	-3.630	0.0434

2.4.5.3 Proportionate change in total biomass in drought and irrigated plots

Associated hypothesis:

- H2.4 Reduction in above-ground biomass under the drought treatment will be proportionally similar to an increase in above-ground biomass under the enhanced rainfall treatment.

Proportionate change in biomass between drought and irrigated treatments were compared, to assess if biomass would vary in similar proportions to reflect the variation in received

precipitation under these two treatments (being c. +50%,.-50%) (Figure 2.40). Summarised across all blocks and nitrogen treatments, the net change in total biomass compared with that in ambient plots was proportionately greater in drought plots in both experimental years (Table 2.35), and so Hypothesis 2.4 was not supported..

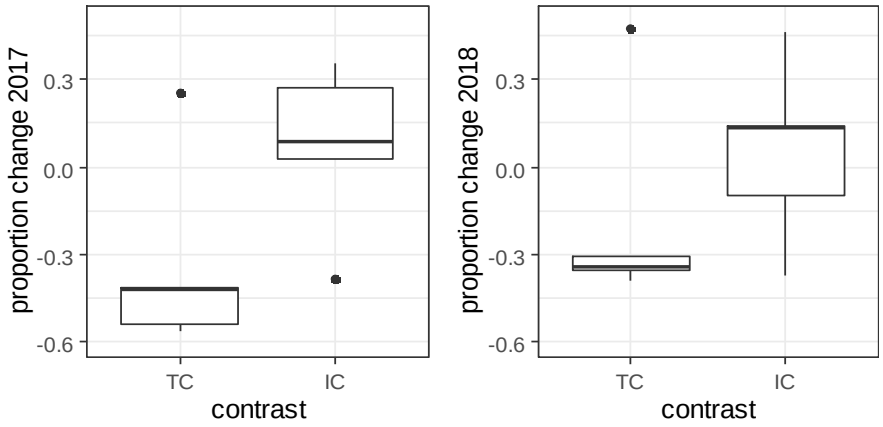


Figure 2.40 Proportion change in biomass from drought and irrigated plots compared with that from ambient plots, 2017 and 2018. Contrast TC is drought, contrast IC is irrigated.

Table 2.35 Proportion change in total biomass from drought and irrigated plots compared with ambient.

date	contrast	median	mean	SD
2017	drought	-0.420	-0.339	0.337
	irrigated	0.090	0.073	0.290
2018	drought	-0.343	-0.185	0.372
	irrigated	0.137	0.054	0.311

Spatial heterogeneity across the site was captured at block level (Figure 2.41).

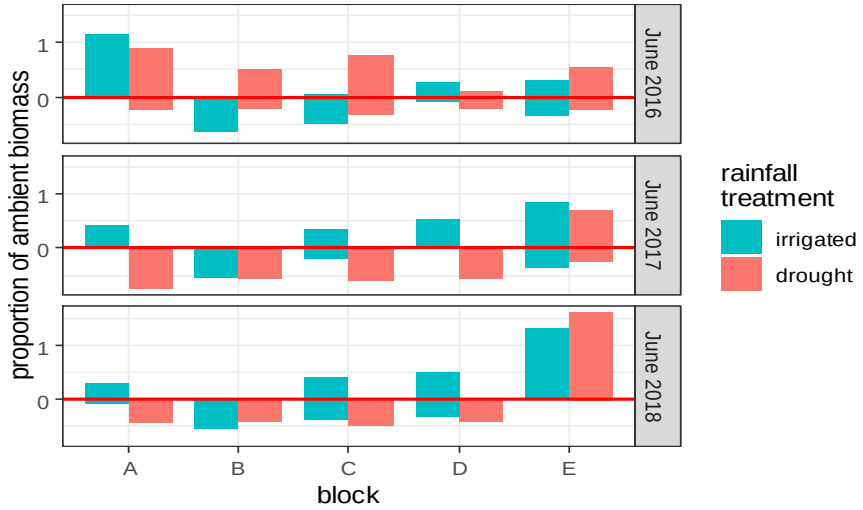


Figure 2.41 Proportion differences in drought and irrigated treatment plots, normalised around ambient biomass (signified by the red line).

2.4.5.4 *Plant group biomass responses to experimental treatments*

Biomass was highest in June 2016 for all plant groups barring legumes (Figure 2.42). Legume biomass was greatest in June 2018, when yield was 47% higher than in the baseline (2016) harvest (Table 2.36).

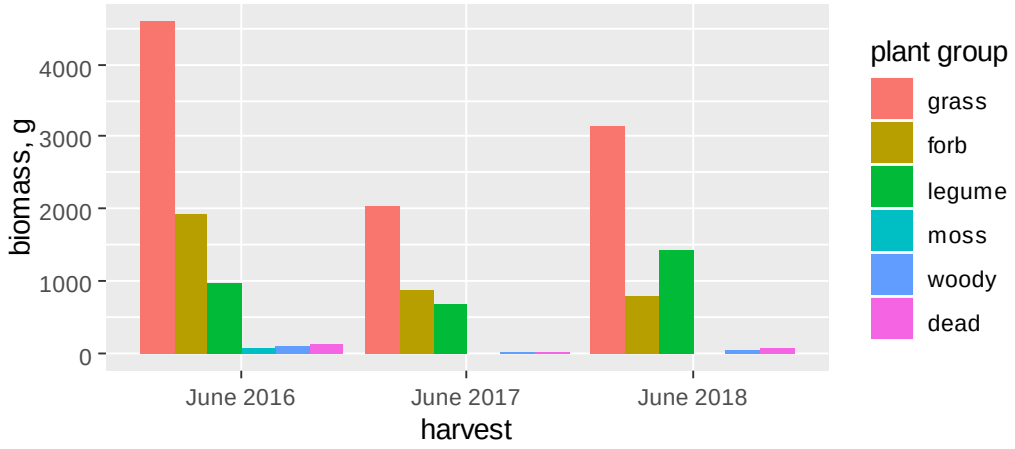


Figure 2.42 Total midseason harvest biomass values for all plant groups.

Table 2.36 Total biomass (g) in each plant group for the three midseason harvests. Mass given is dry weight biomass (g). "Dead" = litter/senesced material retrieved from the sample.

	Plant group yield (g)						**Total harvest (g)**
Harvest	grass	forb	legume	moss	woody	dead	
June 2016	4607.72	1927.5	976.12	74.39	99.08	135.11	7819.92
June 2017	2044.67	873.96	687.09	3.14	40.57	31.6	3681.03
June 2018	3134.23	808.86	1442.27	0.42	52.53	89.76	5528.07

All harvest yields were dominated by grass biomass (Figure 2.43) - 59% of the baseline (2016) midseason harvest; 56% of 2017 midseason; and 57% of 2018 midseason harvest were composed of material from grass, sedge and wood-rush species. Legume species increased their proportion of total biomass year on year and outstripped relative forb productivity in the sampled quadrats in 2018. Moss, woody species and senesced material fractions were consistently very low (<2%); moss and woody species showed a decline in biomass between summer 2016 and summer 2018, while senesced material was more varied in response (Figure 2.44).

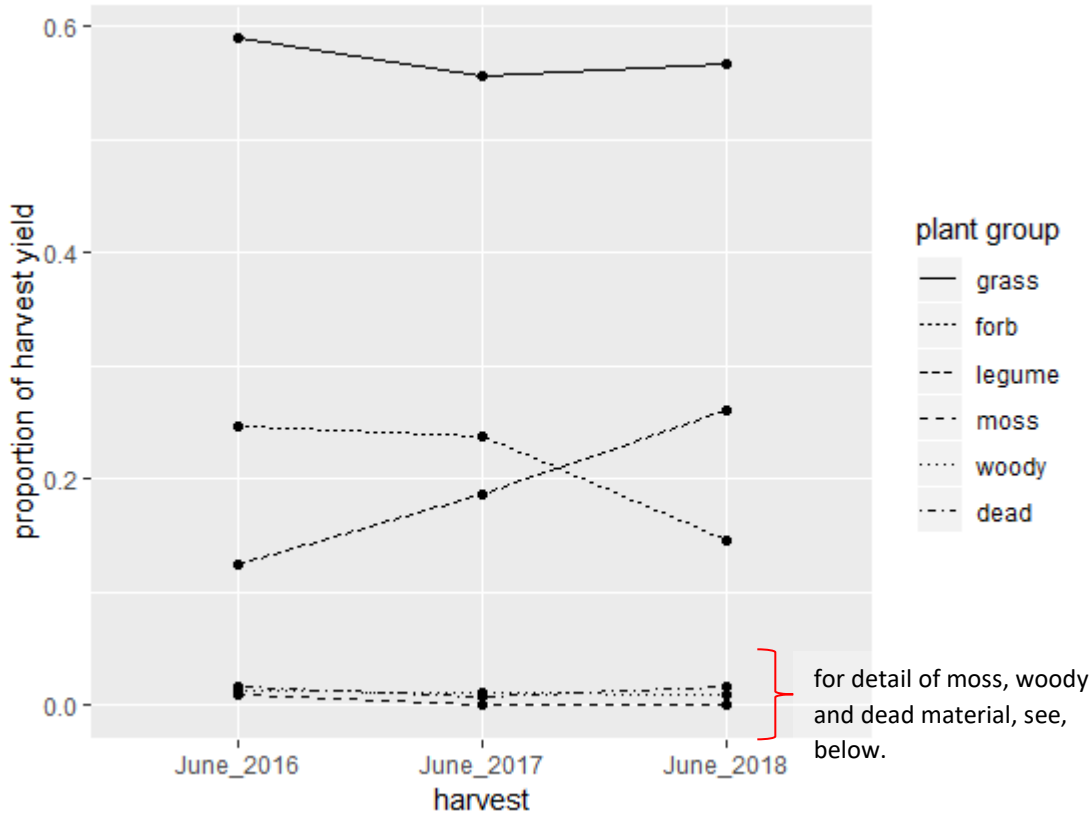


Figure 2.43 Proportion of midseason harvest yield accounted for by each plant group.

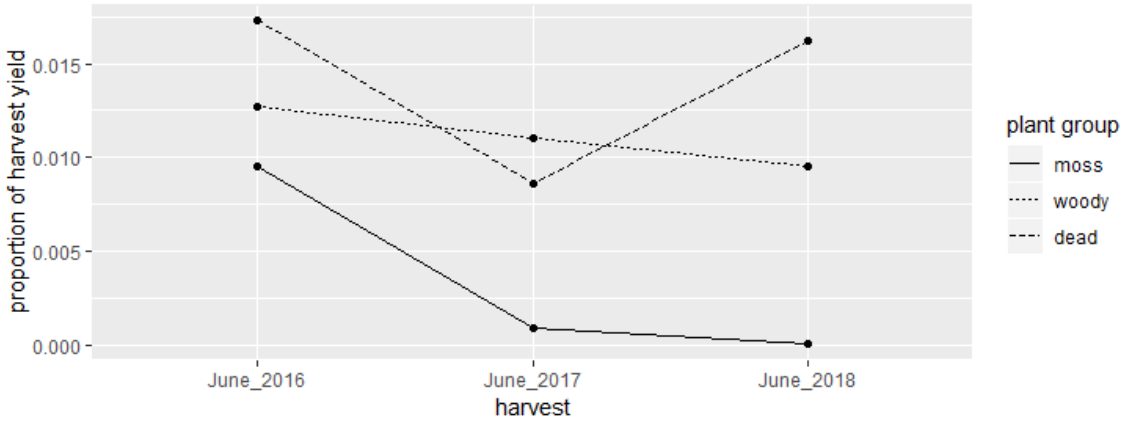


Figure 2.44 Proportion of total biomass accounted for by moss and woody species, and senesced material. Note that the y-axis scale has been expanded in comparison with that in Figure 2.43.

Senesced material declined in samples following the initial harvest in June 2016. As most senescent material comprised grass sheathes, and these were removed in both the June and September mowings, only material standing or newly grown following the Autumn mow could be considered truly senescent by the summer mow the following year. Biomass samples were cut above the ground surface, reducing the potential for senescent material to be present in the sample, though litter presence should be reflected in variations in the litter class in the cover data.

Plant group biomass responses to rainfall treatments were mixed in terms of magnitude and direction. Mean contributions of grass, forb, legume and woody plant groups to total quadrat biomass are given in Table 2.37. The distribution of proportion contributions were similar in 2017 and 2018, and partly reflect historical patterns of proportion seen in the baseline harvest in 2016. Changes in proportionate biomass contributions of the different plant groups were driven predominantly by forb and legume species in 2017 and 2018, whereby legume species consistently increase the portion of legume biomass in the harvested samples (Figure 2.45).

Legume biomass increased proportionately in all rainfall treatments, while the proportion of forb biomass remained relatively constant between the 2016 baseline harvest and June 2017, then was found to have declined across all rainfall treatments by June 2018. The forb fraction was little changed between 2016 and 2017 harvests, then decreased in all rainfall treatments between 2017 and 2018. Forb and legume biomass fractions were negatively correlated across all rainfall treatments in 2017 and 2018 (Spearman's rho); this was significant in ambient control plots in 2017 ($p < 0.05$, Spearman's rho -0.49) and procedural control plots in 2018 ($p < 0.01$, Spearman's rho -0.61).

Table 2.37 Proportion contribution of grass, forb, legume and woody plant groups to total above-ground biomass, in all midseason harvests.

		plant group			
date	**rainfall treatment**	**grass**	**forb**	**legume**	**woody**
June 2016	ambient	0.60	0.21	0.15	0.01
	irrigated	0.61	0.24	0.10	0.01
	drought	0.57	0.31	0.09	0.02
	procedural	0.58	0.23	0.16	0.01
June 2017	ambient	0.54	0.22	0.23	<0.01
	irrigated	0.65	0.21	0.12	0.01
	drought	0.54	0.32	0.10	0.03
	procedural	0.48	0.23	0.27	0.02
June 2018	ambient	0.59	0.11	0.27	0.01
	irrigated	0.63	0.10	0.24	0.01
	drought	0.57	0.24	0.16	0.02
	procedural	0.48	0.16	0.34	<0.01

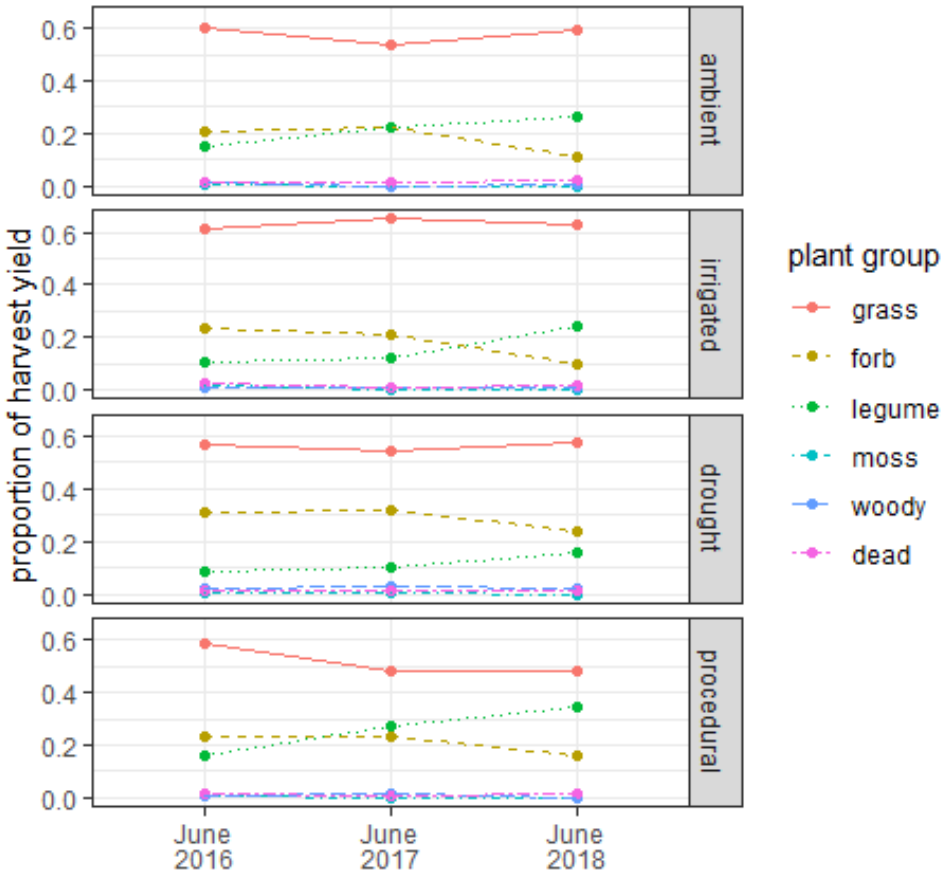


Figure 2.45 Proportionate contribution to total biomass of plant groups, across rainfall treatments.

2.4.5.4.1 Grass biomass response

There was no significant difference in biomass between the rainfall treatment plots prior to the initialisation of the rainfall manipulations. In both 2017 and 2018, grass biomass was highest in irrigated plots and lowest in drought plots (Figure 2.46), with ambient and procedural control biomass being intermediate.

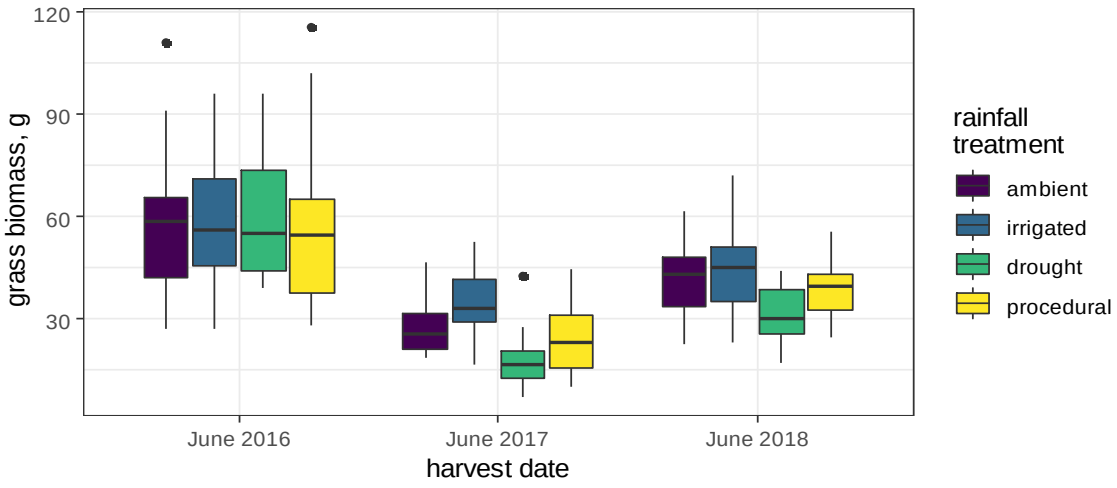


Figure 2.46 Grass biomass response to rainfall treatments, for all midseason harvests.

Linear mixed effect models confirmed that grass biomass was significantly less than in all other rainfall treatments, in both 2017 and 2018 (Table 2.38), when averaged across all levels of nitrogen treatment. There were no significant responses to nitrogen treatment; there was a significant interaction between irrigated rainfall and water-control nitrogen treatment in 2018, but this is really a response to increased water availability rather than a response to nitrogen addition *per se.*

Table 2.38 Significant results of linear mixed effects model for grass biomass response to rainfall and nitrogen treatments. df = Kenward-Roger partial estimation, p-value adjustment is the Tukey method.

model						
response	**estimate**	**SE**	**df**	**t-value**	**p**	
grass biomass 2017 ~ F1 + F2 + F1:F2 + C1 + random						
drought	-0.4924	0.1946	63.00	-2.531	0.0139	*
Significant pairwise comparisons for grass biomass, 2017						
comparison	estimate	SE	df	t-ratio	p	
ambient : drought	0.494	0.0970	59	5.093	<0.0001	
irrigated:drought	0.720	0.0971	59	7.412	<0.0001	
procedural:drought	0.317	0.0971	59	3.258	0.0098	
irrigate:procedural	0.403	0.0970	59	4.158	0.0006	
drought_none:irrigate_Nox	-0.794	0.194	59.0	-4.090	0.0114	
drought_none:irrigate_water	-0.725	0.194	59.0	-3.736	0.0323	
ambient_Nox:drought_water	0.730	0.194	59.0	3.759	0.0303	
irrigate_Nox:procedural_Nox	0.708	0.194	59.0	3.649	0.0412	
irrigate_Nox:drought_Nox	0.807	0.198	59.6	4.074	0.0118	
irrigate_Nox:drought_Nred	0.744	0.194	59.0	3.829	0.0248	
irrigate_Nox:drought_water	0.985	0.194	59.0	5.073	0.0004	
drought_Nox:irrigate_water	-0.739	0.198	59.6	-3.727	0.0330	
irrigate_Nred:drought_water	0.854	0.194	59.0	4.399	0.0043	
irrigate_water:drought_water	0.916	0.194	59.0	4.719	0.0015	
grass biomass 2018 ~ F1 + F2 + F1:F2 + C1 + random						
drought	-16.6646	6.1701	59.22	-2.701	0.0090	**
irrigated:water	18.3199	8.8061	59.40	2.080	0.0418	*
Significant pairwise comparisons for grass biomass, 2018						
comparison	estimate	SE	df	t-ratio	p	
ambient : drought	0.3071	0.0775	59	3.962	0.0011	
irrigated:drought	0.3732	0.0776	59	4.810	0.0001	
procedural:drought	0.2266	0.0776	59	2.920	0.0248	
drought_none:ambient_Nox	-0.6051	0.155	59.0	-3.900	0.0201	
drought_none:irrigate_Nox	-0.6309	0.155	59.0	-4.068	0.0122	
drought_none:ambient_Nred	-0.5791	0.157	59.3	-3.687	0.0370	
drought_none:irrigate_water	-0.6670	0.155	59.0	-4.301	0.0059	

2.4.5.4.2 Forb biomass response

Forb biomass showed little response to either rainfall or nitrogen treatments (Figure 2.47), though the drought treatment response in 2018 was near-significant (t(63) = 1.676, p = 0.0987). Kruskal Wallis one-way ANOVA indicated that forb biomass split clearly into two groups in 2018, with pairings between drought and procedural control plots, and irrigated and ambient control plots. This was partially identified by least squares means contrasts following a linear mixed effects model, which found the irrigation:procedural control contrast in 2018 as being significantly different (irrigated:procedural 2018: estimate -0.4467, SE = 0.149, 59 df, t-value = -2.989, p = 0.0207*), and the irrigation:drought contrast as not significant at p<0.5 level (irrigation:drought 2018: estimate -0.3861, SE = 0.155, 60.5 df, t-value = -2.498, p = 0.0703).

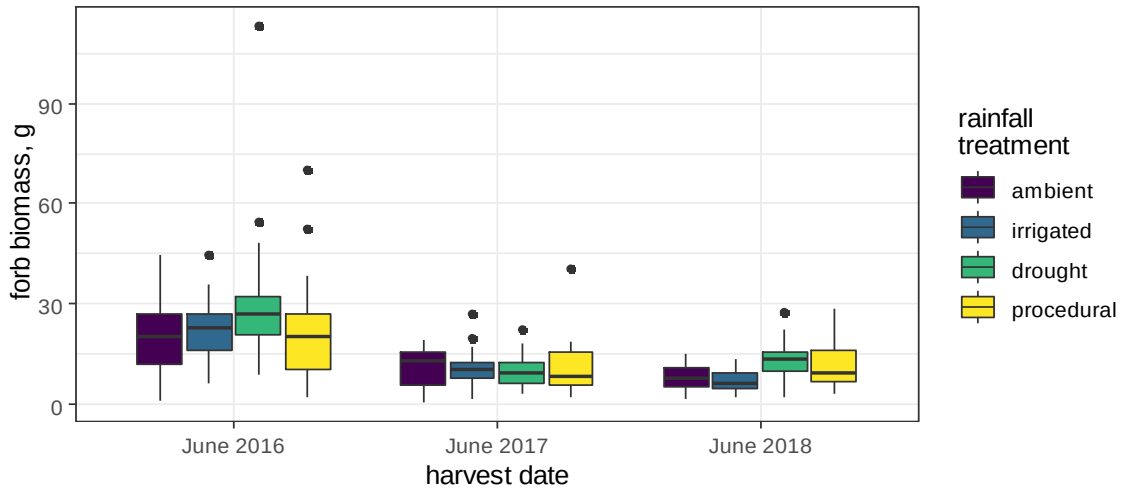


Figure 2.47 Forb biomass response to rainfall treatment.

Although forb biomass did not have a significant response to the nitrogen treatments, the between-group contrast between Nox and Nred were significant in 2018 (Nox 2018:Nred 2018; estimate 0.4059, SE 0.149, 59.1 df, t-value = 2.716, p = 0.0419), with quadrats receiving Nox additions returning greater forb biomass than those receiving Nred additions (Figure 2.48).

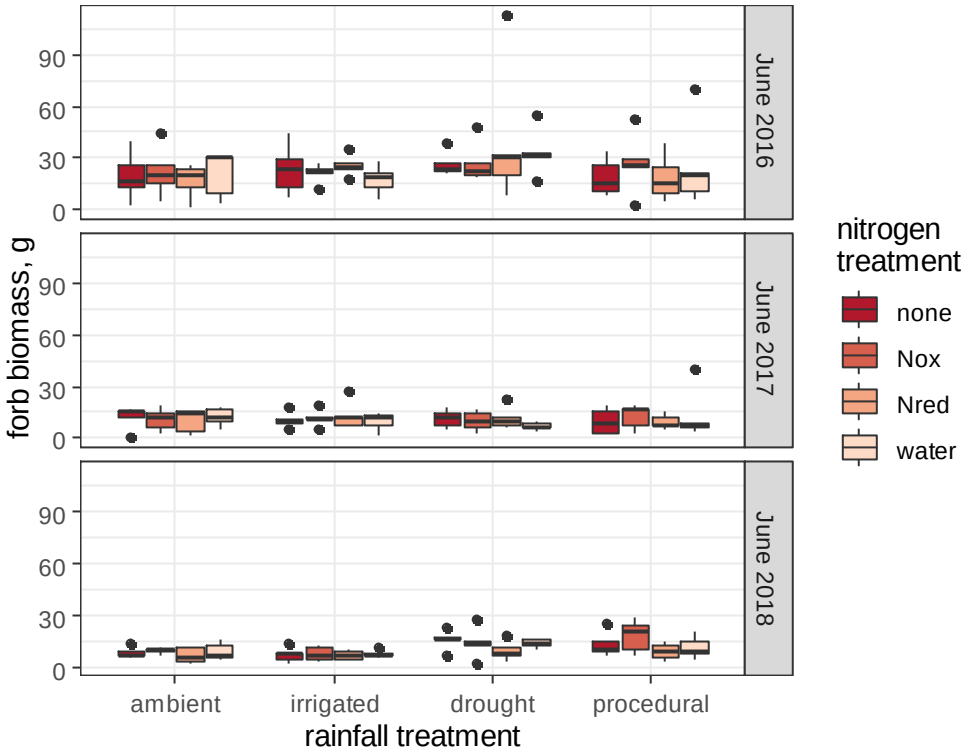


Figure 2.48 Forb biomass response to rainfall and nitrogen treatment combinations.

2.4.5.4.3 Legume biomass response

Legume biomass response was closely linked to water availability; no nitrogen treatment contrasts were significant, when averaged across all rainfall treatments. Biomass was significantly reduced under the drought treatment in both 2017 and 2018; in 2017, biomass was also significantly reduced under the irrigated treatment (Table 2.39), where grass biomass was significantly increased. In both treatment years, mean legume biomass was greatest in the procedural control treatment, and least under the drought treatment (Figure 2.49).

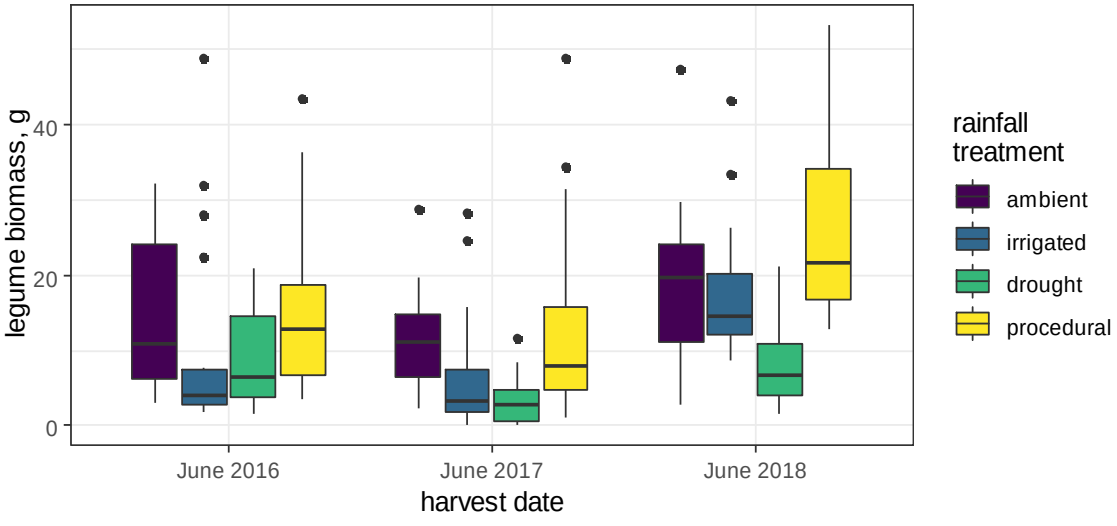


Figure 2.49 Legume biomass response to rainfall treatment.

The drought:water-control interaction was identified as significant (Table 2.39), further signposting the important role of water availability; the addition of even the small amount of water (without additional nitrogen) in the water-only control plots was sufficient to drive a significant increase in biomass compared with the ambient:none plots (which are the comparator levels for rainfall and nitrogen treatments so far as a two-way interaction is concerned), even under conditions of a general drought, as seen in 2017.

Table 2.39 Significant results of linear mixed effects model for legume biomass response to rainfall and nitrogen treatments. df = Kenward-Roger partial estimation, p-value adjustment is the Tukey method.

model						
response	**estimate**	**SE**	**df**	**t-value**	**p**	
legume biomass 2017 ~ F1 + F2 + F1:F2 + C1 + random						
irrigated	-0.9561	0.3747	63.00	-2.552	0.0132	*
drought	-1.3031	0.3751	63.00	-3.474	0.00093	***
drought : N_water	1.2515	0.5285	63.99	2.368	0.0210	*
Significant pairwise comparisons for legume biomass, 2017						
comparison	estimate	SE	df	t-ratio	p	
ambient – irrigated	0.511	0.190	59.8	2.687	0.0449	
ambient – drought	0.913	0.191	60.0	4.769	0.0001	
procedural – drought	0.790	0.193	60.2	4.101	0.0007	
ambient-none:drought_Nox	1.3775	0.375	59.2	3.674	0.0384	
ambient_Nox:drought_Nox	1.3579	0.375	59.2	3.619	0.0446	
legume biomass 2018 ~ F1 + F2 + F1:F2 + C1 + random						
drought	-0.9157	0.3007	58.96	-3.045	0.0035	**
Significant pairwise comparisons for legume biomass, 2018						
comparison	estimate	SE	df	t-ratio	p	
ambient – procedural	-0.413	0.150	59.0	-2.757	0.0378	
ambient – drought	0.632	0.153	59.4	4.116	0.0007	
irrigated – drought	0.754	0.150	59.0	5.037	<0.0001	
procedural – drought	1.044	0.154	59.4	6.763	<0.0001	
procedural_none:drought_none	1.4181	0.306	59.3	4.638	0.0019	
drought_none:procedural_Nox	-1.3168	0.300	59.0	-4.393	0.0044	
drought_none:procedural_Nred	-1.433	0.303	59.2	-4.728	0.0014	

continued over ...

... Table 2.39 continued	estimate	SE	df	t-ratio	p
drought_none:procedural_water	-1.229	0.307	59.3	-4.010	0.0145
procedural_Nox:drought_Nox	1.226	0.300	59.0	4.092	0.0113
drought_Nox:procedural_Nred	-1.342	0.302	59.1	-4.437	0.0038
drought_Nox:irrigated_water	-1.163	0.299	59.0	-3.886	0.0210
drought_Nox:procedural_water	-1.138	0.306	59.3	-3.723	0.0334

2.4.5.4.4 Woody species biomass response

Most records of woody species in survey quadrats were of tree seedlings which did not survive to the following year; of the persistant woody species on site, *Crataegus*, *Rubus* and *Rosa* species were the only ones present in a more mature form. *Crataegus* had been present on site previous to RainDrop being installed, and several areas of mature shrubby growth had been cleared prior to the rainshelter construction. Although the experimental plots had been sited to avoid large stands of shrubby vegetation, there were nonetheless small scattered *Crataegus monogyna* individuals that persisted in the sward. *Rosa* and *Rubus* species were consistently found in fewer quadrats year on year from 2016; *Crataegus* was found in fewer quadrats in 2018 compared to 2016 (Table 2.40).

The observed decline in abundance of the three main woody species on site is reflected in changes in woody biomass from the three midseason harvests (Figure 2.50).

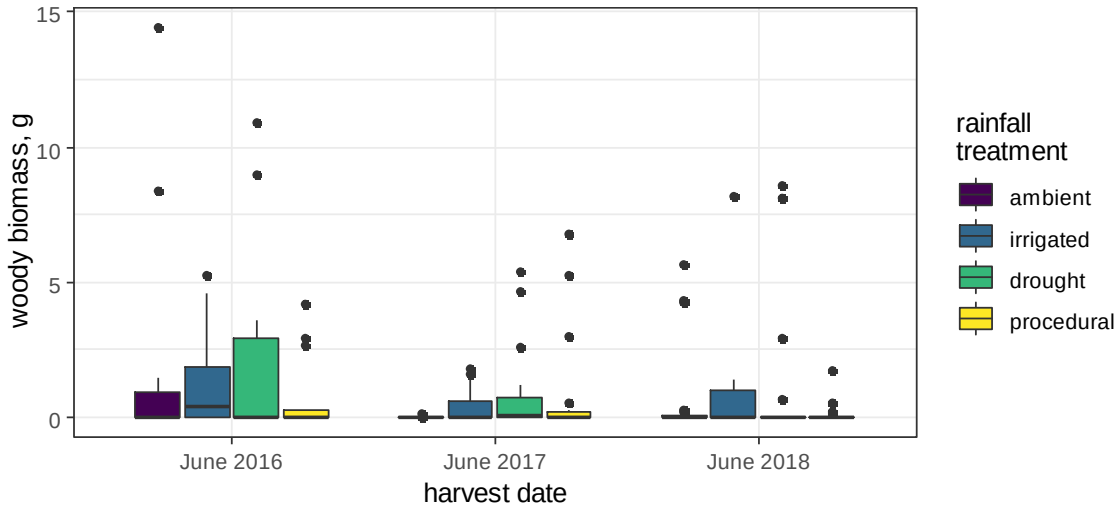


Figure 2.50 Woody species biomass response to rainfall treatments.

Table 2.40 Incidence of woody species in survey quadrats, 2016 to 2018. "cover" is total species cover in that rainfall treatment, in that year.

		2016		2017		2018	
rainfall treatment	**species**	**cover (m^2)**	**no. of quadrats**	**cover (m^2)**	**no. of quadrats**	**cover (m^2)**	**no. of quadrats**
ambient	*Crataegus sp.*	0.11	6	0.23	6	0.08	5
	Rosa sp.	0.06	3	0.02	2	0.03	1
	Rubus agg.	0.04	3	0.08	3	0.03	2
irrigated	*Crataegus sp.*	0.10	8	0.21	9	0.08	5
	Rosa sp.	0.07	4	0.04	2	0.01	1
	Rubus agg.	0.22	10	0.24	8	0.03	3
drought	*Crataegus sp.*	0.09	9	0.20	10	0.16	7
	Rosa sp.	0	0	0	0	0	0
	Rubus agg.	0.06	4	0.06	3	0.03	2
procedural	*Crataegus sp.*	0.09	7	0.16	7	0.09	6
	Rosa sp.	0.01	1	0	0	0	0
	Rubus agg.	0	0	0	0	0	0

2.4.5.4.5 Moss response

The moss component of biomass samples dramatically declined following the first harvest in June 2016 (Figure 2.51); this was expected, as harvests were not made to ground level, so only moss growing above the cut line should be potentially included in the sample.

As the ground surface is uneven, some cuts were made accidentally into the underlying moss layer at times, especially where anthills and other disturbances caused abruptly higher areas, so the moss biomass data is considered unrepresentative of moss presence; percentage cover is perhaps, in this instance, a better indicator of moss abundance.

Proportionately, moss biomass and cover values in treatment years (i.e. 2017 and 2018) showed similar patterns, in that both were highest in ambient plots in 2017 and irrigated plots in 2018, with lowest cover and biomass values in the procedural control plots (Table 2.41).

Table 2.41 Proportion of total annual moss cover and biomass in each rainfall treatment.
Key: = highest value, = lowest value for that year.

	proportion of annual moss cover				proportion of annual moss biomass			
year	**ambient**	**irrigate**	**drought**	**proc**	**ambient**	**irrigate**	**drought**	**proc**
2016	0.41	0.35	0.05	0.20	0.27	0.40	0.19	0.14
2017	0.29	0.28	0.25	0.18	0.40	0.22	0.27	0.11
2018	0.19	0.37	0.31	0.14	0.24	0.57	0.12	0.07

Absolute moss biomass values declined annually from 2016 on; biomass was significantly greater in June 2016 than in either of the following summer harvests ($p<0.001$), which showed a decrease year-on-year. Total moss biomass recovered from all quadrats in June 2016 was 74.4 g; total moss biomass in June 2017 was 3.14 g, and 0.42 g in 2018. Though moss biomass was much reduced in 2017 compared to the previous year, it did show a significant response to the rainfall treatments, with most of the recovered biomass coming from ambient control plots (1.25 g, being 39.8% of the total moss biomass) (see insert, Figure 2.51). Drought plots provided 27.4%, irrigated plots 22.3%, and procedural control plots yielded 10.5% of moss biomass harvested in June 2017.

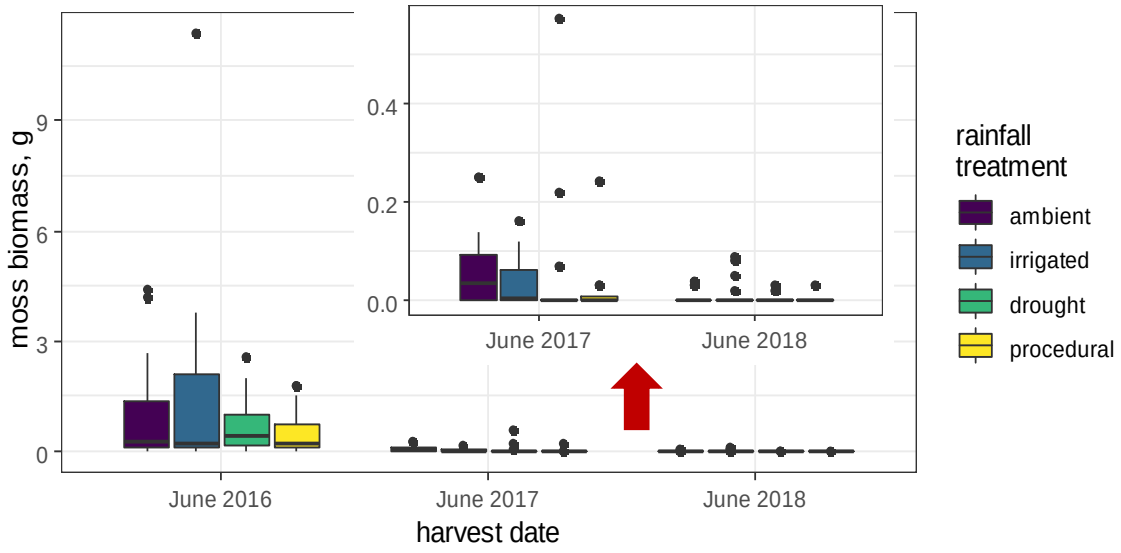


Figure 2.51 Moss biomass response to rainfall treatments. Insert shows biomass results for midseason harvests in 2017 and 2018 on an exploded y-axis, to illustrate year-on-year decrease in moss biomass through the course of the experiment.

Rainfall treatment was not a significant factor for moss biomass in harvested samples in 2018, nor were the relative proportion yields in the same magnitude order: ambient plots 23.8%, irrigated plots 57.1%, procedural controls 7.1%, and drought plots 11.9%.

2.4.5.4.6 Litter response

Rainfall treatment was not a significant factor in litter cover in 2017, but was in 2018 (Figure 2.52). Litter cover in 2018 was significantly greater in drought plots (mean 0.11 m^2) compared to litter in procedural control plots (mean 0.06 m^2) ($p < 0.01$); litter cover in ambient and irrigated plots was intermediate, with mean litter areas of 0.10 m^2 and 0.08 m^2 respectively. Litter cover and biomass had a generally negative relationship: in the two experimental years, litter cover was greatest in the drought plots, which returned the lowest portion of litter biomass of the four rainfall treatments. In the first year following treatment imposition (2017), litter cover was lowest in ambient control plots, where litter biomass was greatest; the difference in litter cover was not significantly influenced by rainfall treatment. The following year saw lowest litter cover in procedural control plots, though greatest biomass being retrieved from irrigated plots (Table 2.42).

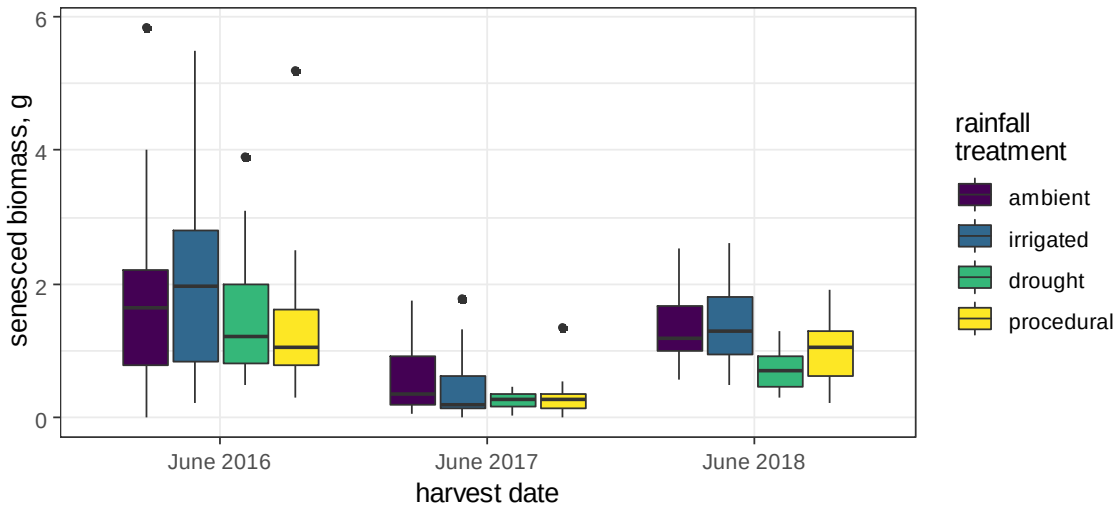


Figure 2.52 Litter area cover (m^2) across the four rainfall treatments.

Table 2.42 Litter area cover and biomass summaries for the baseline (2016) and experimental years (2017 and 2018). Key: = highest value, = lowest value for that year.

	proportion of annual litter cover				proportion of annual litter biomass			
year	**ambient**	**irrigate**	**drought**	**proc**	**ambient**	**irrigate**	**drought**	**proc**
2017	0.19	0.22	0.34	0.25	0.36	0.27	0.17	0.20
2018	0.29	0.23	0.32	0.17	0.30	0.31	0.17	0.22

2.4.5.4.7 Bare ground

For both treatment years, most bare ground was observed in the drought plots, and least in the procedural control plots. The amount of bare ground was significantly associated with rainfall treatment in every June survey (all $p < 0.01$). More bare ground may have been present in 2016 under the drought shelters due to disturbance during construction and on-site works, so only results from 2017 and 2018 are considered to be linked with the imposed rainfall treatments.

Prior to RainDrop being constructed, there was an abundance of large anthills across the grassland within the bounds of Upper Seeds, which were often associated with a local increase in bare ground. Mann-Whitney-Wilcoxon test of two independent samples indicated that quadrats with anthills present had significantly greater mean area of bare ground than quadrats without anthills ($p < 0.001$), after the rainfall treatments had been turned on (Table 2.43).

The anthills were identified as being the work of *Lasius flavus*, the Yellow Meadow Ant (Tim King, pers. comm.), which are known to influence both the vegetation and the amount of bare ground, through their soil reworking activity and the action of their "farmed" aphids on grass overall (King, 1977; Morey, 2010). In 2016, nine survey quadrats contained anthills, of which three persisted into 2018. Of the six anthills noted during the 2018 survey, only one was considered new that season; two had persisted from 2017, and three had been present in 2016 (Table 2.44).

Table 2.43 Mean quadrat bare ground (m^2) for rainfall treatments, and for quadrats with associated anthills within rainfall treatments. No anthills were recorded within any irrigated plots. Following imposition of rainfall treatments, bare ground was consistently greater in drought plots, and least in procedural plots, in all survey years.
. Key: = highest value, = lowest value for that year.

	mean quadrat bare ground (m^2)				**mean bare ground in quadrats with anthills (m^2)**			
year	ambient	irrigate	drought	proc	ambient	irrigate	drought	proc
2016	0.003	0.003	0.015	0.004	<0.0001	NA	0.037	0.015
2017	0.005	0.004	0.024	0.003	0.023	NA	0.048	0.010
2018	0.011	0.024	0.031	0.010	0.040	NA	0.093	0.020

Table 2.44 Incidence of anthills in rainfall treatment plots, across all survey years. "x" indicates anthill present in quadrat for that survey.

block	rainfall treatment	quadrat	present 2016	present 2017	present 2018
A	procedural	nwse	x		
A	drought	senw		x	
A	drought	nesw	x	x	
A	drought	sese	x		
B	procedural	swne		x	
B	procedural	nesw		x	
B	drought	nwnw	x	x	x
B	drought	swne		x	x
C	ambient	nwne		x	
C	ambient	sesw		x	
C	ambient	nwsw	x	x	x
C	drought	nwnw	x	x	x
C	drought	nese	x		
D	procedural	nwsw		x	x
D	procedural	nwne			x
D	drought	swnw	x		
E	procedural	swnw	x		

Factors beyond rainfall treatment that may influence the amount of bare ground include animal disturbance and microfaunal activity. There are no exclusion fences around the site, so wild grazing and other activity of rabbits and deer are uncontrolled; although rabbit droppings were observed in many locations across the site during fieldwork, only one quadrat was found to show major signs of rabbit activity at the time of survey (2017, block D, irrigated plot quadrat sese).

The highest area of bare ground was recorded in drought plots for both experimental years; bare ground in irrigated plots was more extensive in irrigated plots in 2018 than it had been in previous years. It was conjectured, but not tested, whether this increase in bare ground in 2018 may be due to the wetter conditions in irrigated plots driving faster litter decomposition rates. This was not investigated further, but future investigations into the effect of the rainfall treatments on the activity of soil organisms and decomposition rates would be a valuable contribution to developing understanding of processes driving change on these grasslands.

2.5 Discussion

2.5.1 RainDrop: platform effectiveness

The rainfall interception and redistribution system has been shown to produce significant changes in soil moisture, with minimal maintenance or input (Figure 2.8, Figure 2.9, Figure 2.10, Table 2.3). The system was robustly constructed, and procedural flaws encountered in the first three operational years were addressed and rectified. The data acquired from the first three seasons, including the baseline pre-experiment season in 2016, should form a solid foundation for future work on the site. The Raindrop platform has high potential as a tool for assessing changes in the plant community at Upper Seeds under controlled precipitation conditions. There remain questions about the effect of disturbance caused by the experiment construction and the change of management from grazed to mown, and the resulting potential for instability in the plant community, at least in the first few years. Such instability may affect the direction taken by the community in response to both the experimental treatments and prevailing climate conditions. Comparisons of community changes under climatic manipulation at two calcareous grassland sites (Buxton, Derbyshire, and Wytham, Oxfordshire) found the more fertile, early-successional grassland to be more responsive to climate change (Grime *et al.*, 2000). An overall loss of annual species was seen in ungrazed control plots in Gibson's grazing experiment on an adjacent part of Upper Seeds (Gibson, 2010), and has been attributed here to the same cause, i.e. the change of management from being sheep-grazed. Though Gibson's plots remained unmanaged and the RainDrop site management was by bi-annual mowing, they shared the lack of microsite creation caused by animal treading and disturbance that would otherwise provide regeneration and colonisation sites for annual species.

This study focussed on above-ground processes, while recognising that above-and below-ground activities cannot be considered in isolation. There is an acknowledged gap in our understanding of below-ground biomass accumulation under different environmental conditions such as different levels of water availability, whether driven by temporal variability in rainfall input, or changes in rainfall quantities (Fay *et al.*, 2003; Fiala, Tüma and Holub, 2012). Research into below-ground processes and responses would complete the picture of community-level response to changing environmental conditions, though was not carried out here due to resource and protocol constraints, where it was considered important to minimise disturbance to the sward as much as possible. It is through understanding the processes in both above- and below-ground arenas of community interaction with environmental variables that

we may better identify ecosystem functionality. The data suggest that seasonality in precipitation is important to both ecosystem functioning (in terms of above-ground biomass production) and short-term diversity, and it has been shown that temporal variability in rainfall inputs can have as much impact on soil moisture as changes in precipitation amount with no variation in timing of rainfall events (Fay *et al.*, 2002).

2.5.2 Influence of changing water availability on the plant community

2.5.2.1 Biomass response

Associated hypothesis:

> H2.4 Reduction in above-ground biomass under the drought treatment will be proportionally similar to an increase in above-ground biomass under the enhanced rainfall treatment.

By the end of two full growing seasons, it was seen that the imposed rainfall manipulation treatments had had a significant effect on the plant community. Water availability was confirmed as a major limiting factor for the calcareous grassland at Wytham; this was indicated both by the biomass response to the rainfall manipulation treatments, and also to the more general response to background seasonal precipitation. The irrigated plots, which received a 50% supplement of the natural pattern of rainfall, produced more above-ground biomass compared to the other rainfall treatments in 2017; droughted plots, receiving c. 50% less of the ambient rainfall, had significantly lower above-ground biomass than any other treatment, in both experimental years. Although mean quadrat biomass was seen to fall under the drought treatment in both experimental years (Figure 2.39), there was not an equal and opposite response under the irrigated treatments (Figure 2.40); the hypothesis of equal and opposite biomass responses under the drought and irrigation treatments was therefore rejected. It should be noted, however, that even though the irrigation treatment was adding moisture, the soils at Wytham are relatively shallow (c. 20-30 cm) and may not be holding on to the greater proportion of this added moisture. This would render any additional moisture quickly unavailable to plants.

Grasslands are known to be responsive to annual precipitation (Silvertown *et al.*, 1994; Yahdjian and Sala, 2002), with potential for large increases in productivity under conditions of high water availability (Knapp and Smith, 2001), and this was seen to a degree in the relative reduction in

total above-ground biomass in 2017 compared to the previous year. As total annual rainfall in 2015 had been lower than that in 2016, the 2017 reduction in biomass suggested that seasonality of precipitation was important, and that mid-season biomass fluctuated in line with fluctuations in spring and early summer precipitation (Figure 2.37).

The strongest biomass response to the rainfall manipulations was under the drought treatment, where reduced biomass was primarily driven by an overall reduction in grass biomass (Table 2.37); the proportion of total biomass contributed by the grass plant group continued to dominate the plant community in all four rainfall treatments, in both experimental years (Figure 2.45). There was no consistent pattern of increased biomass in the irrigated plots in the plant groups, though graminoids appeared to be advantaged by the increased water availability in 2017 (less so in 2018). Silvertown *et al.* (1994) felt that grasses are selectively favoured by having a higher proportion of root mass closer to the surface, which would enable them to make use of even small supplements of soil moisture in a dry year. That there was no appreciable difference in mean total biomass of the ambient and irrigated plots in 2018 suggests that the precipitation levels seen over Winter 2017 and into Spring 2018 were sufficient to prompt an increase in overall productivity in this grassland community in line with those considered by Knapp and Smith (2001), but that the supplemental moisture made very little difference under those conditions.

2.5.2.2 Diversity response

Associated hypothesis:

> H2.1 Diversity will show a negative correlation with received rainfall amount, i.e. will be higher under the drought treatment

No correlation was found between overall species richness and above-ground biomass, at quadrat, block or treatment scale, in any of the three years. This was contrary to findings from many other studies (Naeem *et al.*, 1994; Hector *et al.*, 1999; Weisser *et al.*, 2017), but it is not unknown for this relationship to be unclear, to take one of a number of forms (Kelemen *et al.*, 2013), or, indeed, not be present (Adler *et al.*, 2011). The sole correlation between any expression of diversity and biomass was when diversity was considered in terms of plant groups; legume diversity was found to be significantly correlated with total quadrat biomass in 2017 ($p < 0.5$), though no other plant group or measure (e.g. C-S-R strategy) was associated with biomass. It should be remembered, however, that the experiment was only carried on over two

full seasons, which may have been too brief for the community to reach a new equilibrium within the rainfall treatments. There is a chance that diversity and productivity may show a relationship in the future, given sufficient time for it to establish.

In contrast to Gibson's results from grazing experiments on an adjacent part of Upper Seeds, all the species (n = 9) permanently lost from the surveyed quadrats on RainDrop between June 2016 and June 2017 were perennials (one legume, three grass, and five forb species). Not counting tree seedlings, eight species (three annual, five perennial) were present in survey quadrats in June 2016 and June 2018, but not in June 2017, though all but one (*Sonchus oleraceus*, a ruderal annual) were still present in the general site context that year. This indicated a general contraction of range for those seven species, which may be sensitive to factors such as a different seasonality of rainfall. Functional C-S-R class responses mostly fit with expectations, with a net increase in abundance of stress-tolerant species under the drought treatment between the baseline in 2016 and the survey in 2018, while their relative cover decreased in the irrigated plots. There was a shift in the abundances of the more competitive species away from the drought plots and into the other rainfall treatments (Figure 2.35), where they would have benefitted from relatively higher soil moisture.

It is likely that the change in management from being sheep-grazed to a biannual hay cut has impacted on the community beyond the influence of the rainfall treatments, and altered its successional status. Management techniques are acknowledged to have a strong effect on species composition and richness in grasslands (Bakker, 1989; Jacquemyn, Brys and Hermy, 2003; Stevens *et al.*, 2011; Kormann *et al.*, 2015; Bonari *et al.*, 2017) and this is of major importance when considering conservation management for species diversity (Bullock *et al.*, 1994; Fagan *et al.*, 2008; Maalouf *et al.*, 2012). When considering the effect of environmental manipulations on the Wytham grasslands, Gibson (1986) considered management practice to be the most important influence on species loss, with potential to affect the conservation value of species-rich calcareous grasslands (Grime *et al.*, 2000).

The most widely used conservation management for grasslands are grazing and cutting for hay (Ravenscroft, Fridley and Grime, 2014), each of which may favour particular species or groups of species. Grazing and cutting regimes limit the dominance of highly competitive, productive species; slower low-growing species are more able to tolerate repeated cutting or grazing, and are not excluded due to being shaded out by tall faster-growing species. Stress tolerators are poor competitors, and where grazing or mowing is abandoned, may be soon lost from the community if successional progress to scrub is allowed to take place. Biannual mowing, as has

been adopted across the RainDrop site, has been found to be useful in maintaining diversity in mesic grasslands (Bennie *et al.* 2006; Maalouf *et al.* 2012), though the combined effect of biannual mowing coupled with increased drought is not fully understood at present (Maalouf *et al.* 2012). Mowing in this way, where all the arisings are removed from site, reduces potential nitrogen inputs into the soil (Gowing *et al.*, 2002), and though reduced productivity reduces the size and value of a hay crop (Manchester *et al.*, 1999), the resulting lower nutrient status has been linked to increased biodiversity. Grazing may affect soil through compaction and disturbance, providing ephemeral opportunities for ruderal colonisation, and the lack of this provision by stock may have only been partially offset on the RainDrop site by the activities of deer, rabbits and ants.

2.5.2.3 Changes in community composition

Associated hypotheses:

H2.2 Grass to forb abundance ratio will be greater under the enhanced rainfall treatment compared to that under the drought treatment.

H2.3 More competitive species will increase in abundance under the enhanced rainfall treatment compared to that under the drought treatment.

Despite the short timescale of the experiment, some changes were seen in community composition with regard to relative abundance and productivity of plant groups. The legume biomass fraction increased over time across all rainfall treatments, with a corresponding decline in proportion of biomass contributed by forb species (Figure 2.45, Table 2.37); legume and forb cover proportions were also inversely correlated across all rainfall treatments in 2017 and 2018. Mean forb biomass was significantly greater under both sheltered treatments (drought and procedural control) in 2018, compared to the irrigated and ambient controls, which suggests that forb species were able to take advantage of reduced grass productivity under both sheltered treatments, but does not explain the decline in graminoid biomass. The decline in graminoid biomass may be linked to a contraction in *Trisetum flavescens* abundance across the site after 2016, which is a CSR generalist species with conservative requirements for moisture and soil nutrients, and has a moderately high tolerance of disturbance. It is known that grass species experience can vary at a local scale through cyclic surges and contractions, and this may have been the case for *Trisetum*.

As the plant community is water-limited, there was an expectation that the additional moisture available under the irrigated treatments would increase competition and thereby reduce diversity in these plots. This was not seen, and species diversity (as measured by Simpson's Index of Diversity) in irrigated plots was comparable to that in the ambient control plots in both years of experimental rainfall manipulation, while species diversity increased in the drought plots (Figure 2.15). Functional diversity, measured with respect to Grime's C-S-R groups, did, however, increase in irrigated plots in 2017 relative to the other rainfall treatments (Figure 2.16), so whereas species diversity did not show a response, there was a measurable effect of increased water availability on the community's functional composition. The increase in Grime's C-S-R group diversity supports the argument functional diversity may be as or more informative than species diversity when considering community-level responses to changing environmental variables.

2.5.3 Influence of nitrogen addition treatments on the plant community

Associated hypothesis:

> H2.5 Biomass will increase in response to nitrogen addition, where moisture availability is sufficient. Where soil moisture is insufficient for nitrogen-uptake, nitrogen addition will have no effect on plot biomass.

The pre-existing community on Upper Seeds was species rich and diverse in terms of plant functional groups and Grime's C-S-R strategies, which theoretically should render it more resistant and resilient to environmental perturbations (Grime *et al.*, 2000). Over the three survey seasons, there were changes in the plant community in terms of productivity, diversity and abundance of species and functional groups (plant groups, life histories, C-S-R classes), not all of which could be related to the imposed rainfall manipulation treatments. The low detection rate of a response to nitrogen addition (of either form) suggests that any such response was small, and masked by the much greater response to variation in soil moisture provided by the rainfall treatments. The addition dose rate was low, and only applied over the growing season, but, in addition to the background deposition of atmospheric nitrogen, would have increased total nitrogen load above the critical level of 15-25 kg N ha^{-1} $year^{-1}$ (Bobbink and Hettelingh, 2011). It is thought that long-term nitrogen deposition in excess of the critical load leads to fundamental impacts on calcareous grasslands, through increased dominance of tall grasses and a decline in diversity through increased mineralisation, nitrogen-leaching and soil

acidification. The nitrogen treatment may not have been sufficiently long to allow a nitrogen signal to be seen in any of the metrics considered.

2.5.4 Summary

In order to get a better picture of community compositional response, it is necessary to make observations over a longer timeframe than the three seasons encompassed by this research, and hence why long-term experimental platforms like RainDrop are crucial to understanding the processes involved, particularly when there is potential for lag-effects to have a significant impact (Van Looy, Lejeune and Verbeke, 2016; Weisser *et al.*, 2017). The platform design was trialled over the three seasons 2016-2018, and design flaws that became apparent have been addressed. Principal among these was the realisation that the sloping roof panels on the procedural control shelters were channelling some precipitation away from the research plot below and onto the ground along the east and western edges, thus causing a droughting effect on the plot. By orienting the roof panel into a horizontal position rather than an inclined one, this unintentional interception was prevented in the subsequent summer. Other potential influences of the rainshelter structure itself have been minimised through the selection of appropriate materials (Yahdjian and Sala, 2002), and recent research into microclimate impacts suggests that such shelters produce little or nothing in the way of non-drought effects on above-ground primary production (Loik *et al.*, 2019).

The combination of change of management from grazing to mowing, the early-reversion status of the on-site vegetation and the perturbations caused by the imposed rainfall manipulations resulted in complex responses from the existing plant community, the reasons for which were often unclear. In the short term, it may be that management is more important than climate in directing community change. Two years is a short timescale in which to see changes at a community level, but the small divergence seen in community composition between the drought and irrigated treatment plots - particularly in terms of relative plant group and C-S-R class abundances - suggest that rainfall amount, and its seasonality, are important environmental factors driving community change in these grasslands. Short-term responses to nitrogen treatment were undetectable, but there is scope within the RainDrop experiment to continue these additions, in order to better assess longer term responses to the interaction of rainfall and nitrogen deposition.

Chapter 3 The interaction of soil depth and nitrogen deposition on a model grassland community

In this chapter, I consider the roles of soil depth and nitrogen deposition in driving plant productivity and, consequentially, as potential determinants of community composition in lowland calcareous grasslands.

The following abbreviations and contractions have been used throughout the text and figures:

- where species' names have been contracted to genus in figures and text for brevity, they refer to the three species *Lotus corniculatus*, *Dactylis glomerata* and *Silene vulgaris*.
- "shoot" denotes above-ground biomass
- "root" denotes below-ground biomass
- Nox denotes oxidised nitrogen treatment ($NaNO_3$)
- Nred denotes reduced nitrogen treatment (NH_4Cl)

3.1 Introduction

Nitrogen and water are major limiting resources in many terrestrial ecosystems (Boring *et al.*, 1988; Nordin *et al.*, 2000; Bobbink *et al.*, 2010). They form the foundations of plant physiological processes, and are critical for growth, development and reproduction. Water is an essential component of photosynthesis and facilitates nutrient uptake, transport and distribution within plants. Nitrogen is essential for the formation of proteins, enzymes and amino acids. It is a major component of chlorophyll and the enzyme Rubisco, which facilitates the conversion of carbon dioxide to carbohydrates as the first step in photosynthesis. The main routes whereby nitrogen can enter terrestrial ecosystems are through atmospheric deposition of organic and inorganic forms of nitrogen, biological fixation of organic nitrogen derived from animal and plant residues, and bacterial fixation of molecular N_2, particularly in association with legumes.

Most nitrogen held in the soil is in plant-unavailable, organic forms; these are converted to plant-available, inorganic forms through mineralisation by free-living and plant-symbiotic micro-organisms. Complex organic compounds are changed into ammonium and then into nitrates through this mineralisation process. Mineral nutrients are absorbed through plant roots as ions in solution; nitrogen as either nitrate (NO_3^-, i.e. the oxidised form) or ammonium (NH_4^+, the reduced form of nitrogen). The different forms of nitrogen are more easily taken up by plants under conditions of different soil pH – ammonium is more readily acquired under low pH, whereas nitrate is predominantly utilised under basic or calcareous soils. Where ammonium is taken up directly by plants, it can be used immediately in the formation of proteins. The rate of mineralisation depends on soil water content, temperature, and soil pH; the activity of soil organisms is retarded by low temperatures and dry conditions. Dry soils, such as the shallow, well-drained soils underlying calcareous grasslands, experience reduced mineralisation rates and hence, a reduced amount of plant-available nitrogen.

There is a wide acceptance that deposition of atmospheric nitrogen presents a serious threat to the structure and functioning of sensitive ecosystems (NEGTAP, 2001; Sheppard *et al.*, 2008; Stevens *et al.*, 2009; Maskell *et al.*, 2010; Ladwig *et al.*, 2012; Phoenix *et al.*, 2012; Payne *et al.*, 2013; Southon *et al.*, 2013; Field *et al.*, 2014). In a context of anthropogenic nitrogen enrichment, empirical and experimental evidence shows that an increase in available nitrogen often results in increased biomass (Tilman, 1987; Bobbink *et al.*, 2010; Phoenix *et al.*, 2012) and reduced species richness (Stevens *et al.*, 2006, 2011, 2016; Maskell *et al.*, 2010; Payne *et al.*, 2013; Soons *et al.*, 2017) in natural and semi-natural grassland habitats. Higher levels of

available nitrogen generally lead to an increase in the upper fraction of plant biomass (hereinafter referred to as "shoot"), with a proportional reduction in relative root biomass (Cahill, 2002; Lamb, Kembel and Cahill, 2009); in nutrient and water-poor environments, the converse has been found, i.e. that plants allocate proportionately more biomass to roots for increased nitrogen acquisition (Titlyanova *et al.*, 1999; Callaway, Pennings and Richards, 2003; Hermans *et al.*, 2006; Weißhuhn, Auge and Prati, 2011; Poeplau, 2016). Competitive species with rapid growth rates are generally widely variable in their root:shoot response (Chapin, 1980b), whereas species characteristic of stressful environments show reduced plasticity in their biomass allocation patterns (Grime, 1977). Where no or little effect of experimental nitrogen addition has been seen, both in controlled and field experiments, this has been interpreted as indicative that other resources such as phosphorus are primary or co-limiting in these cases (e.g. Phoenix *et al.*, 2012).

As well as influencing the growth and development of different plant structures, variations in nutrient availability have an effect on plant tissue chemistry. Nutrient uptake strategies are often linked to storage capacity (Chapin, 1980b); carbon and nitrogen can be used immediately to fulfil growth or functional requirements, or stored against future need. Storage of nitrogen frees a plant from day to day reliance on acquiring an adequate supply from the soil reservoir, and stored reserves can be between 25-70% of the total plant nitrogen (Chapin, 1980b, 1980a). The availability of soil nitrogen is a main driver of plant carbon-nitrogen balance, and plants growing under nitrogen enrichment are expected to have higher tissue nitrogen content than plants from less fertile conditions (Chapin, 1980b; Heyburn *et al.*, 2017), and reduced plant tissue C:N ratio (Meunier *et al.*, 2017; Van de Waal *et al.*, 2018). Nitrogen concentration has been found to be linearly correlated with growth rate (Ågren and Franklin, 2003), whereby faster-growing species have been found to have higher total nitrogen concentration, with more nitrogen allocated to leaves, and a higher photosynthetic nitrogen-use efficiency (Poorter, Remkes and Lambers, 1990).

C:N ratios are a useful tool in considering plant nutrient status and changes in the proportional allocation of these nutrients to different plant tissues, as above- and below-ground organs are involved in different processes, though care must be taken to separately consider changes in concentration (i.e. % of nutrient) and relative proportions of nutrients (e.g. C:N ratio). The general observed pattern is for an increase in both carbon and nitrogen content (% carbon and % nitrogen) with improved nutrient acquisition, but with a relatively higher increase in tissue nitrogen, which results in a reduced C:N ratio. This pattern has been seen in both laboratory conditions, e.g. Du *et al.* (2014) and open-field research, e.g. Heyburn *et al.* (2017), though Luo

*et al. (*2017) found the opposite response along geographical nitrogen gradients in southern Chinese grasslands. Whereas Heyburn *et al.* (2017) found an increase in % nitrogen in both shoot and root tissue, as well as an increase in % carbon in shoot (but not root) tissue, and a decrease in both shoot and root C:N ratios, Luo *et al.* (2017) found a positive relationship between C:N ratio and nitrogen deposition that was tempered by a negative relationship between C:N ratio and soil moisture availability. Contradictory to theory, relative nitrogen allocations shifted from roots to shoots with declining soil moisture, and C:N increased, i.e. there was a greater proportionate amount of carbon to nitrogen; this was attributed to changes in biomass partitioning in favour of structural support tissues with relatively higher carbon but lower nitrogen content, such as stems and coarse roots.

While nutrient limitation commonly leads to an increase in the proportion of root biomass to shoot biomass, water limitation (water stress) prevents this reallocation of resources, through a direct inhibition of photosynthesis and retardation of other metabolic processes (Weißhuhn, Auge and Prati, 2011). Water stress directly affects plant growth and development, and there is a general tendency in all terrestrial biomes towards a reduction in plant biomass under drought conditions (Sala *et al.*, 1988; Titlyanova *et al.*, 1999; Rouphael *et al.*, 2012). Most studies on the effect of drought have focussed on shoot biomass, and there is an accepted positive association between water availability and shoot net primary production (Sala *et al.*, 1988; Knapp and Smith, 2001). Reduced water availability has been widely seen to result in reduced shoot biomass (Sala *et al.*, 1988; Silvertown *et al.*, 1994; Yahdjian and Sala, 2002; Frank, 2007; Grime *et al.*, 2008; Fiala, Tüma and Holub, 2012). Grasslands have high below-ground productivity; Hui and Jackson (2006) found the root portion of total phytomass to range from 0.40 to 0.86 across 12 global sites, and Titlyanova *et al.* (1999) recorded root fractions of total phytomass not less than 0.70 across 10 Siberian grasslands. The picture is less clear for below-ground productivity than it is for the above-ground portion and, as a consequence, for total plant biomass in these habitats (Titlyanova *et al.*, 1999; Fiala, Tüma and Holub, 2012; Hui *et al.*, 2018).

As seen above, there are many studies of the effects of changing nitrogen deposition or precipitation levels on grassland communities (Silvertown *et al.*, 1994; Lane, Coffin and Lauenroth, 1998; Lane *et al.*, 2000; Hui and Jackson, 2006; Zhou, Talley and Luo, 2009). Although soil depth controls soil moisture and nutrient availability, and influences root architecture and development, the influence of soil depth as a determinant of productivity in natural and semi-natural grassland plant communities has been less frequently considered. At a landscape scale, soil depth has been found to have a positive effect on plant nutrient uptake and shoot productivity, for example in an open-field experiment located on serpentine

grassland in California (Selmants, Zavaleta and Wolf, 2014); similar results were returned for a field-based manipulation of soil depth in a tallgrass prairie community (Dornbush and Wilsey, 2010), which found evidence that soil depth influenced species composition and shoot biomass. In contrast, studies at the Buxton Long Term Climate Research Laboratory in Derbyshire, UK, (Fridley *et al.*, 2011) have shown how very small-scale heterogeneity in soil depth may contribute to overall community stability, through highly localised variation in nutrient and soil moisture, and small differences in the effect of other environmental factors such as seasonal and diurnal changes in temperature. Such variations alter the competitive playing field at a small scale, allowing the expression of greater number of species-species interactions, perhaps at an individual plant level. Studies of plant performance in green roofs have found substrate depth to have the most significant effect on plant development (Gabrych, Kotze and Lehvävirta, 2016; Ondoño, Martínez-Sánchez and Moreno, 2016); these plant communities are located on exposed, shallow substrates, and, in the UK, often comprise species associated with calcareous grasslands.

Soil depth acts as a resource reservoir, with deeper soils buffering against potential nutrient and moisture deficits over seasonal droughts (Fridley *et al.*, 2011), and therefore it seems reasonable to expect a measurable physiological response by the plant community to differing soil depth. Moisture and nutrient availability are, in part, controlled by the simple volume of soil available to foraging roots for resource acquisition; a larger volume provides a larger spatial niche, with an inherently greater amount of nutrients and soil moisture. Dimensionality within this niche space is important; plants have different rooting depths and vary in the way they use above-ground space (Cahill, 2002; Hodge, 2004; Selmants, Zavaleta and Wolf, 2014). These are expressed in their root foraging strategies and root architecture (Berendse, 1981; Dornbush and Wilsey, 2010) - rooting depth is often considered a trait related to water-stress tolerance, whereby deeper roots confer greater drought tolerance (Castillo *et al.*, 2017) - and their above-ground growth habit, e.g. low and sprawling, or slender and upright. The importance of differential foraging habits via varying vertical distribution of root biomass was ably demonstrated by Dimitrakopoulos and Schmid (2004); they considered the volume of soil available, rather than only depth, as a factor in determining plant performance, and found that, though plant height linearly increased from shallow to deep soil, other biomass traits (such as stem and leaf biomass) responded differently, reflecting observed differences in mean depth of each species' root biomass.

The primary limiting factor for a species or community will change as conditions and requirements change with season and life stage (Meyer-Grünefeldt *et al.*, 2015; Tulloss and

Cadenasso, 2016) and from year to year. Environmental factors do not, however, act in isolation. This interaction of factors is difficult to untangle through field observation and open-field experiments, and mesocosm experiments have been used successfully to further understanding of the underlying processes at work in complex situations (Dunnett and Grime, 1999; Buckland and Grime, 2000; Dukes, 2001; Van den Berge *et al.*, 2014; Hui *et al.*, 2018). Simplified model communities are useful for investigating specific questions about community responses to varying environmental conditions (Dunnett and Grime, 1999; Buckland and Grime, 2000). By limiting the number of member species in a model community, interactions that are complex in the natural state can be explored. Such experimental communities allow for controlling of environmental factors less easily manipulated in the wild, and although there are issues regarding the scaling-up of observed effects to a wider community or landscape level, if an interaction cannot be observed in a simplified model community, then it is unlikely to be a significant element of processes at work in natural habitats (Gibson *et al.*, 1999).

In order to investigate the relative importance of soil depth and nitrogen availability as limiting factors for calcareous grassland species, a mesocosm experiment was set up with simplified communities comprising representative members of three main plant groups (grasses, legumes, herbs). Differentiation by depth in terms of root foraging offers niche partitioning through the soil profile and confers benefits to both shallow and deep-rooting strategies. Most soil nutrients are found within the top c. 20 cm (Jobbágy and Jackson, 2001; Selmants, Zavaleta and Wolf, 2014), which encompasses both soil depths used in the mesocosms. Shallow roots are able to access soil moisture sooner after rainfall or watering (von Felten *et al.*, 2012); while this upper part of the soil profile remains sufficiently moist, nutrients are also therefore more readily accessed by shallow roots. It is thought that deep roots are advantageous in drought conditions (Castillo *et al.*, 2017), and are able to draw on and redistribute soil moisture from further down the soil profile.

Characteristic plant species of calcareous grassland communities are adapted to seasonally low soil moisture and generally low nutrient levels, which makes these communities vulnerable to increased nutrient inputs, e.g. as a result of agricultural improvement or nitrogen components in atmospheric pollution. Such species are expected to show increased biomass under nitrogen enrichment when sufficient water is available, but to have a relatively reduced biomass in water-limited situations. It has already been seen that there is a general pattern of increased total plant biomass with increased water availability in terrestrial ecosystems, and I hypothesised that total biomass in the mesocosms would show a similar positive relationship with soil depth (H3.1). I also hypothesised that species adapted to a low nutrient environment

would show a positive response to nitrogen addition, and that species' biomass would show a positive response to a nitrogen-addition treatment (H3.2). As calcareous grasslands occur on soils with high pH, it was further hypothesised that species characteristic of this habitat would be adapted to more readily absorb the oxidised (nitrate) form of nitrogen rather than a reduced form (ammonium), and that this would be expressed as increased biomass (H3.3).

Resource optimisation theory suggests that plants allocate resources to maximise biomass production, i.e. to those structures that are involved in acquiring the most limiting resource (Johnson and Thornley, 1987; Korner and Renhardt, 1987; Tilman, 1987; McCarthy and Enquist, 2007); plant productivity is expected to be more limited by nitrogen availability as water availability increases to optimum. Where soil moisture and nutrients are not limiting, this theory predicts an increase in shoot biomass with increased nitrogen availability, in order to maximise photosynthate production. Conversely, functional equilibrium between shoot and root fractions would promote a relative increase in root biomass to improve supply of soil-based resources (McConnaughay and Coleman, 1999). Thus, water stress would be indicated by an overall reduction in total plant biomass reflecting an inhibition of nutrient uptake and photosynthesis, along with a relative increase in the root fraction. I hypothesised that the root:shoot ratio would reflect changing biomass allocation depending on water and nutrient status, having a lower value in mesocosms that received nitrogen enrichment (H3.4). Linked to this is an expectation that plants grown in nitrogen-enriched conditions would have greater tissue nitrogen, due to increased availability and absorption of nutrients (H3.5).

In summary, hypotheses tested were:

H3.1 Biomass will show a positive relationship with depth.

H3.2 Biomass will increase with nitrogen enrichment.

H3.3 Biomass will show a stronger positive response to oxidised nitrogen (Nox) than to reduced nitrogen (Nred) enrichments.

H3.4 The root:shoot ratio (R:S) will decrease a) in deeper soil, and b) with nitrogen addition.

H3.5 Tissue nitrogen content will be greater in nitrogen-enriched conditions.

3.2 Methods

3.2.1 Experiment design

Thirty mesocosms were arranged in a randomised block design (five blocks of six mesocosms, (see Figure 3.2) with an approximate north-south orientation); gaps between blocks were of the order of 1 m. Within each block, three bins were each assigned to shallow (8 cm) and deep (20 cm) soil conditions. These depths provide 10 litres and 25 litres respectively, of available growing medium. The two soil depths were chosen to reflect the natural range of soil depth under calcareous grasslands in the UK (Tansley, 1939; Cranfield University, 2018). Bins were further randomly assigned to one of the three nitrogen-addition treatments; oxidized (as $NaNO_3$) or reduced (as NH_4Cl), or a water addition control in a fully factorial design.

Polyvinyl chloride bins with 0.39 m diameter and 0.58 m height were used; these were sufficiently large to allow the mesocosm communities to develop without undue spatial pressure. Each bin was filled with layers of gravel, sand and a soil-based growing medium (Figure 3.1), as follows:

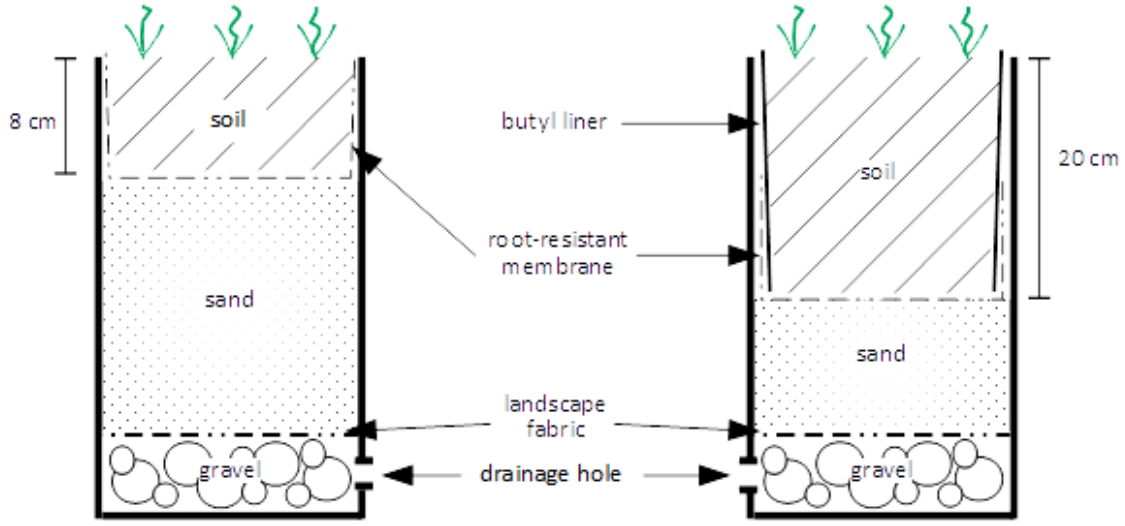


Figure 3.1 Schematic of mesocosm bin layers (not to scale).

Coarse gravel was placed in the base to a level just above the drainage hole. This provided an open drainage zone to prevent potential clogging and aid drainage of overlying layers. Above the gravel, a layer of weed control landscaping fabric was used to hold a layer of fine white sand. This sand acted as a soil moisture reservoir and ensured a standard depth of hydrologically connected soil/sand through the bins, regardless of soil depth variation. The fine sand was capped by a further water-permeable membrane, that was also resistant to root

penetration, and topped with the growing medium to the appropriate experimental depth (8 cm or 20 cm). A collar of black butyl liner was used in the deep bins, to prevent roots accessing the media below the soil by growing over the top of the membrane.

The growing medium was prepared by mixing coarse sand 1:1 with soil taken from the agricultural weeds plot at Wytham Woods Estate. This plot lies on calcareous Elmton I series soils, and is managed for rare agricultural weeds; it had received no fertiliser input for eight years to the date of collection, but had been regularly turned to provide the level of disturbance favoured by the target species there. Using this soil also ensured the mesocosms were inoculated with an appropriate microbial community. Growing medium pH (mean 8.47, n = 5, SD = 0.02) was assessed to confirm that it was within the range of native calcareous soils that support grasslands in the UK (NERC, 2014).

The mesocosm array was located on a level, open area on the Open University's Walton Hall campus, and exposed to ambient weather conditions (Figure 3.2). Drainage holes in the base allowed excess water to exit the system during periods of heavy rainfall, and the mesocosms were watered manually over the growing season if there had been no precipitation for six days. Once the bins had been filled, they were allowed to settle for four weeks before being planted.

Figure 3.2 Layout of mesocosm array.

The mesocosms in August 2017, showing the linear arrangement of the array, and generally open nature of the location on the Open University's Walton Hall campus, Milton Keynes.

North is towards the bottom of the picture. Towards the south, there was a large water tank and a small area of tall predominantly ruderal species.

A mixed hedge c. 6-12 m in height was located c. 15 m east of the array (i.e. to the left of the picture). An open tarmac car park was located immediately to the west (right of the picture) of the mesocosm area.

Photo credit: M. Stone

3.2.2 The plants

Each mesocosm contained a model community comprising three plants each of three perennial species commonly found in UK lowland calcareous grasslands – *Dactylis glomerata* (a grass), *Lotus corniculatus* (a legume) and *Silene vulgaris* (a non-leguminous forb). *Lotus*, *Silene* and *Dactylis* were chosen in order to a) represent three main plant groups found in calcareous grasslands (legumes, non-leguminous forbs and graminoids); and b) represent species with different requirements in terms of soil moisture and nutrient availability (the habitat niche). These requirements are presented below (Table 3.1) as Ellenberg values for the traits fertility, N, and moisture, F:

Table 3.1 Habitat preference matrix for model community species. Ellenberg values for fertility (N) and moisture (F) (Hill *et al.*, 1999).

	Fertility, N	
Moisture, F	High (5-7)	Low (2-4)
Damp (5-6)	*Dactylis glomerata* F: 5, N: 6	
Dry (3-4)	*Silene vulgaris* F: 4, N: 5	*Lotus corniculatus* F: 4, N: 3

All three species exhibit adaptations to increase drought tolerance.

Dactylis glomerata L. (Cock's-foot) is a persistent perennial grass of calcareous grassland and other habitats. It is a cool-season species, being one of the first species into growth in Spring, is fast-growing, and summer-drought tolerant. It has numerous forms, which exhibit varying degrees of plasticity in adapting to local conditions (Zhouri *et al.*, 2017).

Silene vulgaris (Moench) Garcke (Bladder Campion) is a slender perennial herb. It is dioecious and widespread, occurring in nutrient-poor habitats on neutral to basic soils. Adaptations to avoid dehydration include a glaucous, waxy covering, and deeply penetrating roots (Rankou *et al.*, 2015).

Lotus corniculatus L. (Common Bird's-foot Trefoil) is a taprooted perennial legume commonly found in grasslands throughout the UK. The stems are glabrous and sparsely hairy, and the leaves smooth. *L. corniculatus* is drought-tolerant, with long roots, and known for its ability to adapt to adverse conditions.

Lotus and *Silene* were sourced as over-wintered plug plants (British Wildflower Plants, North Burlingham, UK), from seed collected from UK-grown plants. *Dactylis* is a fast-growing grass, which starts into growth early in the season, and in order to prevent early domination of the model communities, *Dactylis* plants were raised in early Spring 2016 from UK-sourced seed (Emorsgate Seeds, King's Lynn, UK) and transplanted out with the plug plants in May 2016.

Plants were placed with the three species as mixed-up as possible, to maximise interspecific competition (Figure 3.3).

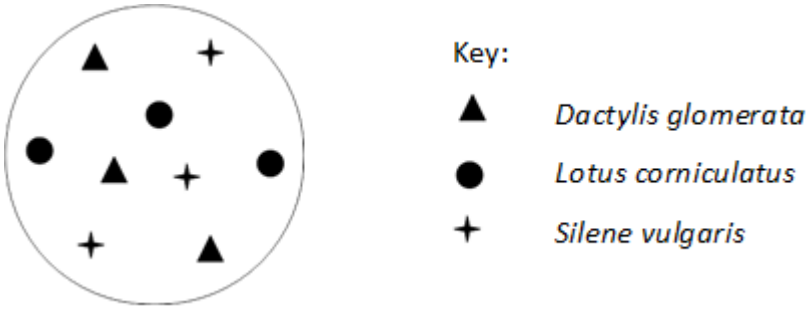


Figure 3.3 Mesocosm planting scheme.

3.2.3 Nitrogen addition treatments

Nitrogen addition treatments commenced in April 2017 and continued monthly through the growing season of 2017 (i.e. April to October), and from March to harvest in July 2018. At each treatment, mesocosms were subject to an addition of one of two aqueous forms of nitrogen (oxidised, as $NaNO_3$, or reduced, as NH_4Cl), and the water control addition was added at the same time and the same volume. Nitrogen addition rates were calculated to approximate 25 kg N ha^{-1} yr^{-1}. As atmospheric nitrogen deposition for the Walton Hall area has been calculated as 18.2 kg N ha^{-1} yr^{-1} (NEGTAP, 2001), this pushed the total nitrogen deposition on the mesocosms beyond the currently accepted critical load for calcareous grassland (i.e. 15-25 kg N ha^{-1} yr^{-1} (APIS, 2018)).

3.2.4 Productivity measures

Aerial cover of each species was assessed in late June 2018. The number of flower spikes/tillers was also counted at this time. All plant material was then harvested, and divided immediately into above- ("shoot") and below-ground ("root") biomass for each species. Plant material for

each species in each biomass class (shoot or root) was measured after drying at 70 ^{o}C for 48 hours.

Levels of shoot and root tissue nitrogen and carbon were determined for all three species, using an Elementar Elemental Analyser Vario EL III (Elementar Gmbh, Langenselbold, Germany). Soil samples were also taken from each mesocosm at the end of the experiment, and soil carbon and nitrogen content assessed in the same way.

3.3 Analysis

All analyses were carried out using packages available in R open-source software (R Development Core Team, 2018). Biomass responses to soil depth and nitrogen treatments were investigated using a linear or generalised linear mixed model approach, with soil depth and nitrogen treatments as fixed effects, and block as a random effect, using the lmer() function in *lme4* package (Version 1.1.456; R Development Core Team, 2018). Where variance was not constant or data normally distributed, a generalised linear mixed model approach was used (function glmer(), also from *lme4* package).

Community and individual species' responses to soil depth and nitrogen treatment as single fixed effects were investigated using the following models (i.e. responses were averaged across all levels of the alternative experimental treatment):

- *for soil depth*: response ~ F1 + R1
- *for nitrogen treatment*: response ~ F2 + R1

Community level and individual species' responses to the interaction of soil depth and nitrogen treatment were investigated using the following general model:

model: response ~ F1 + F2 + F1:F2 + R1

In all the above models, F1 is soil depth, a fixed effect with two levels (deep, shallow); F2 is nitrogen treatment, a fixed effect with three levels (control, oxidised nitrogen (Nox), reduced nitrogen (Nred)); R1 is the random effect of block.

Biomass data were log-transformed prior to inclusion in the models to meet the requirement for normality. Model outputs are included in tabular form, along with any significant pairwise comparisons.

Flowering effort was quantified as the number of individual flowers in *Silene*, of flower heads in *Lotus*, and flower stems in *Dactylis* (Figure 3.4). This approach is similar to that taken by several studies of nectar and pollen resources (e.g. Hicks *et al.*, 2016).

a. Lotus corniculatus flower head *b. Dactylis glomerata flower stem* *c. Silene vulgaris flower*

Figure 3.4 Flower units used in the study. All photos by the author.

Where boxplots have been used to visualise and present data, the following conventions have been adopted:

- midline = median
- box = interquartile range (IQR), i.e. lower and upper quartiles
- upper whisker extends to the smaller of the maximum data value and Q3 + 1.5*IQR
- lower whisker extends to the larger of the smallest data value and Q1 – 1.5*IQR
- any data more extreme than the whisker extent values are plotted individually as small dots.

3.4 Results

Results pertinent to individual hypotheses can be found as follows:

• H3.1 Biomass will show a positive relationship with soil depth	*in sections*	3.4.1 (soil depth), 3.4.3 (soil depth: nitrogen interaction)
• H3.2 Biomass will increase with nitrogen enrichment	*in sections*	3.4.2 (nitrogen treatment), 3.4.3 (soil depth: nitrogen interaction)
• H3.3 Biomass will show a stronger positive response to oxidised nitrogen (Nox) than to reduced nitrogen (Nred) enrichment	*in sections*	3.4.2 (nitrogen treatment), 3.4.3 (soil depth: nitrogen interaction)
• H3.4 The root:shoot (R:S) ratio will be lower a) in deeper soil, and b) with nitrogen addition	*in section*	3.4.4
• H3.5 Tissue nitrogen content will be greater in nitrogen-addition treatments	*in section*	3.4.7

Where model estimate coefficients are given for biomass models, these are on the log, not the response, scale. Axes in figures are on the response scale, unless otherwise stated.

3.4.1 Biomass responses to soil depth

Associated hypothesis:

H3.1 Biomass will show a positive relationship with soil depth

3.4.1.1 Community response

Total community biomass (the sum of above- and below-ground, i.e. shoot and root, biomass) in the mesocosms was greater in those with the deep soil treatment compared to those with shallow soil (Table 3.2, Figure 3.5).

Table 3.2 Descriptive statistics for community biomass responses (g) to soil depth. For all cases, n = 15.

	shallow soil		deep soil	
biomass metric	**mean**	**SD**	**mean**	**SD**
total	92.38	13.62	128.8	12.14
shoot	45.03	6.99	72.53	8.32
root	47.35	9.67	56.26	9.89

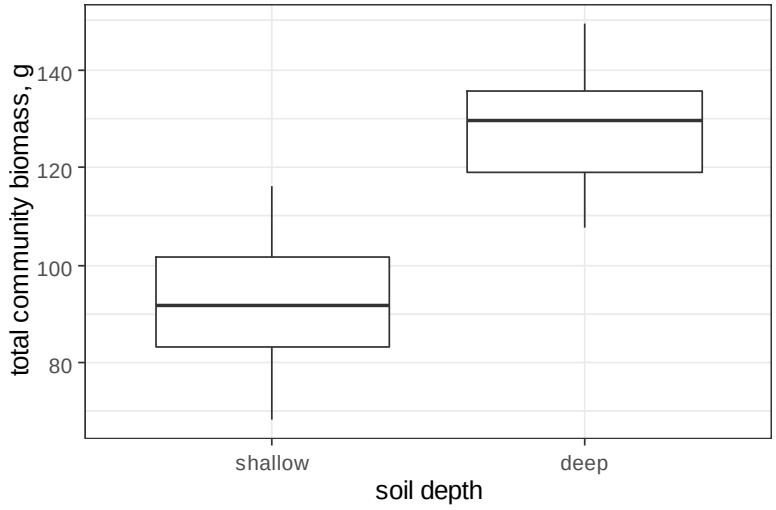


Figure 3.5 Total community biomass response to soil depth treatments.

Community biomass responses to the different soil depths were investigated via a mixed effects model (Table 3.3).

*Table 3.3 Output from mixed effect model of total community biomass to soil depth treatments. Reference level is deep soil treatment. Significance levels are indicated as follows: ^ = p<0.1, * = p<0.05, ** = p<0.01, ***= p<0.001.*

model (community level)						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
total biomass ~ F1 + R1						
shallow	-0.3385	0.0409	28.00	-7.369	$5.03e^{-08}$	***

There was a greater disparity in community shoot biomass between the two soil depths than in root biomass, though both biomass fractions were greater in the deep soil treatment (Figure

3.6). This difference was significant for both shoot biomass and root biomass, when considered across all nitrogen treatments (see Table 3.4).

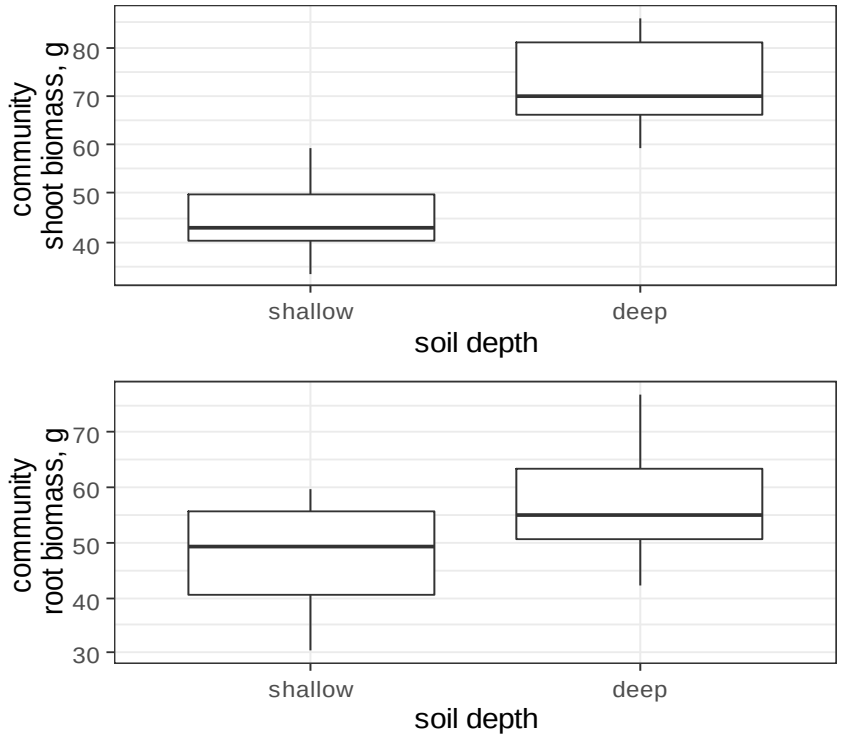


Figure 3.6 Community biomass responses to soil depth, by above- and below-ground fraction (shoot, root).

Table 3.4 Output from mixed effects model - responses of above- and below-ground fractions of community biomass to soil depth treatments. Reference level is deep soil treatment.

model (community level)						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
shoot biomass ~ F1 + R1						
shallow	-0.4817	0.0485	24.00	-9.926	5.68e-10	***
root biomass ~ F1 + R1						
shallow	-0.1795	0.0724	28.00	-2.478	0.0195	*

3.4.1.2 Species responses

All species were more productive in deep soil compared with the shallow soil treatment (Figure 3.7, Table 3.5), with mean total plant biomass being between 20% and 75% higher in the deep soil treatment.

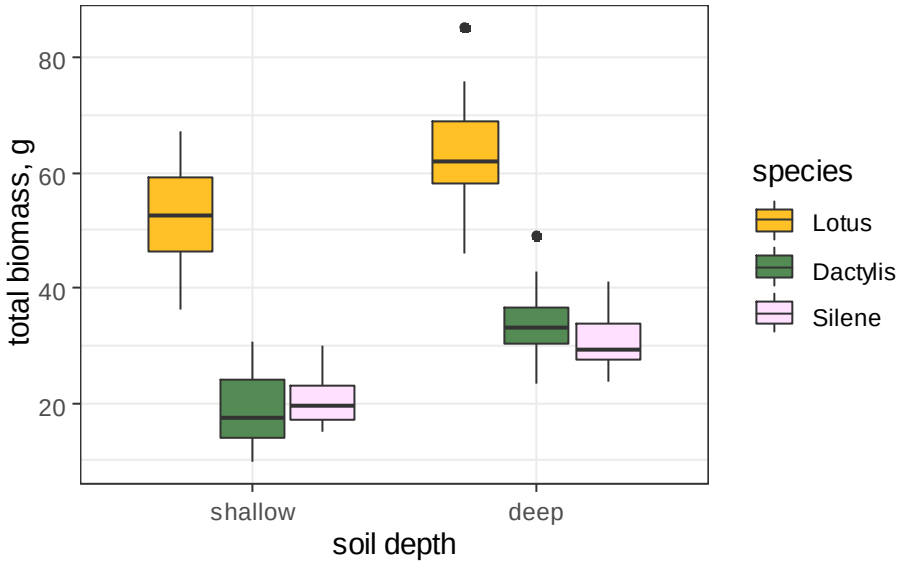


Figure 3.7 Individual species' total biomass responses to soil depth.

Table 3.5 Descriptive statistics for individual species' biomass responses (g) to soil depth treatments, averaged across all nitrogen treatments. For all cases, n = 15.

	shallow soil		**deep soil**	
biomass metric	**mean**	**SD**	**mean**	**SD**
Lotus				
total	52.43	8.91	63.54	9.63
shoot	26.92	4.53	40.36	5.47
root	25.52	6.20	23.19	6.53
Dactylis				
total	19.35	6.92	33.86	6.72
shoot	11.43	4.22	23.56	4.74
root	7.92	3.48	10.30	2.68
Silene				
total	20.60	4.61	31.39	5.24
shoot	6.68	1.87	8.61	2.75
root	13.91	3.05	22.78	4.16

Individual species' responses were investigated using the previously defined mixed effects model, which was applied to each individual species' data separately. Plants in the deep soil treatment were significantly more productive than those in the shallow soil treatment, for each of the three species (Table 3.6). A general positive trend in *Dactylis* biomass was noted from Block 1 to Block 5, with biomass values becoming measurably greater from south to north along

the array, though not significantly so at p<0.05 level. Neither *Lotus* nor *Silene* showed this response, and it was accounted for in the model through the inclusion of *block* as a random effect (R1).

Table 3.6 Output from mixed effects models of total biomass in nitrogen-control mesocosms to soil depth, for each of the three species. Reference level was the deep soil treatment.

model (species level)						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
total *Lotus* biomass ~ F1 + F2 + F1:F2 + R1						
shallow	-0.1957	0.0597	28.00	-3.276	0.0028	**
total *Dactylis* biomass ~ F1 + F2 + F1:F2 + R1						
shallow	-0.6027	0.1049	24.00	-5.746	$6.37e^{-06}$	***
total *Silene* biomass ~ F1 + F2 + F1:F2 + R1						
shallow	-0.4314	0.0701	28.00	-6.157	$1.2e^{-06}$	***

Shoot biomass in all species was greater in the deep soil treatment (Figure 3.8).

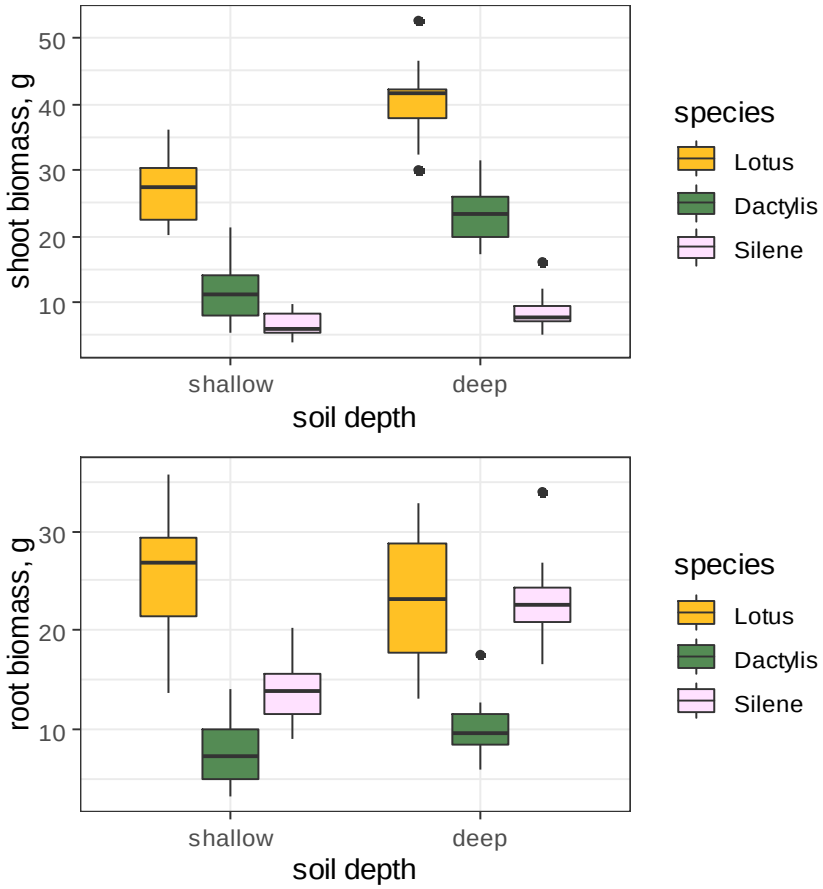


Figure 3.8 Individual species' shoot and root biomass responses to soil depth.

In all three species, shoot biomass was significantly greater in deep soil compared to shallow soil, averaged across all nitrogen treatments. Root biomass had a more mixed response to soil depth: *Lotus* root biomass was greater in shallow soil compared with deep soil (though not significantly so), whereas *Dactylis* and *Silene* mean root biomass was significantly reduced in the shallow soil treatment compared to the deep soil treatment mesocosms (Table 3.7).

Table 3.7 Shoot and root biomass responses of individual species to soil depth, as identified by linear mixed effects model.

model (species level)						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
***Lotus* shoot biomass ~ F1 + R1**						
shallow	-0.4098	0.0563	28.00	-7.273	$6.43e^{-08}$	***
***Lotus* root biomass ~ F1 + R1**						
shallow	0.1036	0.0989	24.00	1.048	0.305	
***Dactylis* shoot biomass ~ F1 + R1**						
shallow	-0.7700	0.1080	24.00	-7.13	$2.27e^{-07}$	***
***Dactylis* root biomass ~ F1 + R1**						
shallow	-0.3259	0.1296	24.00	-2.515	0.019	*
***Silene* shoot biomass ~ F1 + R1**						
shallow	-0.2488	0.1040	28.00	-2.393	0.0237	*
***Silene* root biomass ~ F1 + R1**						
shallow	-0.5005	0.0702	24.00	-7.126	$2.29e^{-07}$	***

Hypothesis H3.1 was therefore supported by the data, with total biomass being greater in the deep soil treatment compared with that in the shallow soil treatment; this held for community (mesocosm) level, and for all individual species, though resource allocation to above-and below-ground biomass varied between species.

3.4.2 Biomass response to nitrogen treatment

Associated hypotheses:

H3.2 Biomass will increase with nitrogen enrichment

H3.3 Biomass will show a stronger positive response to oxidised nitrogen (N_{ox}) than to reduced nitrogen (N_{red}) enrichment

3.4.2.1 Community response

When averaged across both soil treatments, all mean biomass metrics (shoot, root and total) were greater in the nitrogen-addition treatments compared with the control treatment (Table 3.8, Figure 3.9).

Table 3.8 Descriptive statistics for community biomass (g): mean and SD, averaged across both soil depth treatments.

	control		Nox		Nred	
biomass metric	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
total	105.43	26.3	110.77	19.01	115.57	22.64
shoot	55.56	18.57	60.75	17.17	60.04	12.52
root	49.87	10.59	50.02	7.29	55.53	13.25

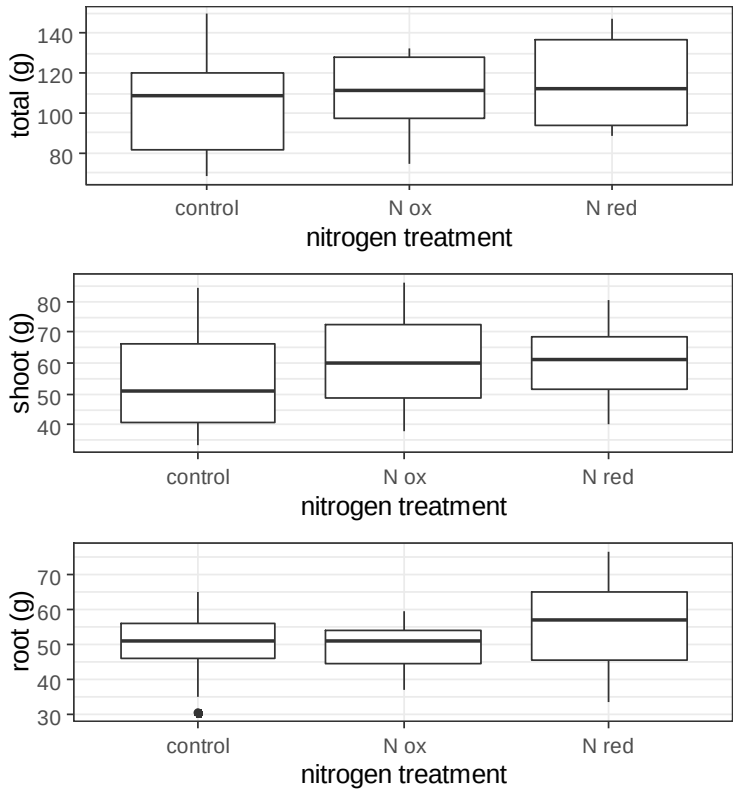


Figure 3.9 Community biomass response to nitrogen treatments.

Mixed effects models indicated that total mesocosm biomass was not significantly influenced by nitrogen treatment, when averaged across all soil depths (Table 3.9) (but see section 3.4.3.1 for changes in response depending on soil depth). Community shoot and root biomass measures were also not significantly different under the three nitrogen treatments, when averaged over

both soil depths, though all biomass metrics were measurably higher under Nox and Nred additions compared with the control.

Table 3.9 Output from mixed effects models of community biomass responses to nitrogen treatments, as identified by mixed effects models.

model						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
Total community biomass ~ F2 + R1						
Nox	0.0640	0.0962	27.00	0.665	0.511	
Nred	0.1031	0.0962	27.00	1.073	0.293	
Community shoot biomass ~ F2 + R1						
Nox	0.1021	0.1271	27.00	0.803	0.429	
Nred	0.1071	0.1271	27.00	0.842	0.407	
Community root biomass ~ F2 + R1						
Nox	0.0162	0.0974	27.00	0.166	0.870	
Nred	0.1032	0.0974	27.00	1.059	0.299	

3.4.2.2 Species responses

Dactylis and *Silene* produced more total biomass with the two nitrogen addition treatments; *Lotus* was least productive under the oxidised N treatments, and most productive under the reduced N treatment (Table 3.10).

Table 3.10 Species' biomass responses to nitrogen treatments, averaged across both soil depth treatments.

	control		**Nox**		**Nred**	
biomass metric	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Lotus corniculatus						
total	58.69	13.07	55.74	7.68	59.54	11.49
shoot	34.03	10.06	32.71	8.94	34.17	6.81
root	24.65	6.96	23.03	6.19	25.38	6.38
Dactylis glomerata						
total	21.66	10.16	28.86	9.51	29.30	9.25
shoot	14.16	7.92	19.83	7.73	18.51	6.58
root	7.50	2.58	9.04	2.67	10.78	3.86
Silene vulgaris						
total	25.09	7.05	26.17	6.78	26.73	8.71
shoot	7.37	3.23	8.21	2.40	7.36	1.89
root	17.72	4.90	17.95	4.72	19.38	7.67

Resource allocation varied between species, with different degrees of increase/decrease in shoot and root biomass with the nitrogen additions (Figure 3.10). *Dactylis* produced more shoot and root biomass under the two nitrogen-addition treatments compared with the control; the increase in total *Silene* biomass under the nitrogen-additions was due to an increase in shoot biomass under Nox, and in root biomass under Nred; the reduction in *Lotus* biomass under Nox was due to a decrease in both shoot and root biomass.

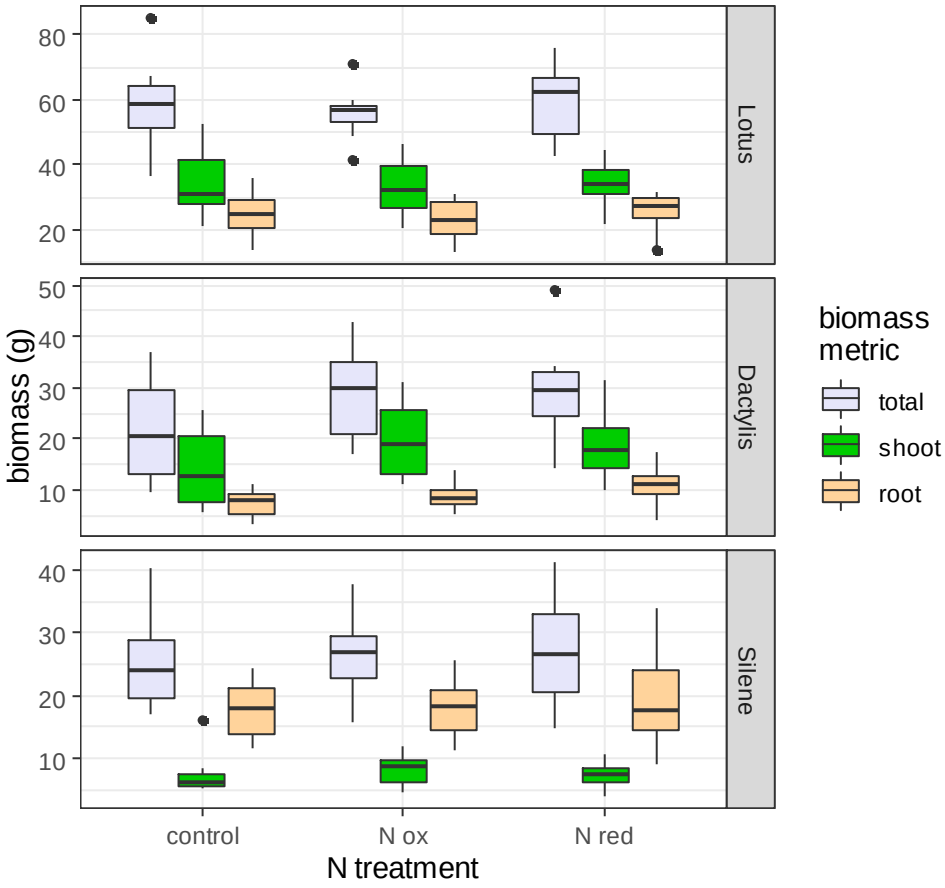


Figure 3.10 Species' biomass responses to nitrogen treatments.

Mixed effects models indicated that *Dactylis* responded most strongly to nitrogen treatment: neither *Lotus* nor *Silene* were found to have significant or near-significant responses to nitrogen treatment, in any biomass measure.

model (species level)						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
***Lotus* shoot biomass ~ F2 + R1**						
Nox	-0.0358	0.1189	27.00	-0.301	0.766	
Nred	0.0235	0.1189	27.00	0.198	0.845	
***Lotus* root biomass ~ F2 + R1**						
Nox	-0.0652	0.1248	23.00	-0.522	0.607	
Nred	0.0314	0.1248	23.00	0.251	0.804	
***Dactylis* shoot biomass ~ F2 + R1**						
Nox	0.4265	0.2101	27.00	2.030	0.0523	^
Nred	0.3711	0.2101	27.00	1.766	0.0887	^
***Dactylis* root biomass ~ F2 + R1**						
Nox	0.2114	0.1660	23.00	1.273	0.2156	
Nred	0.3588	0.1660	23.00	2.162	0.0413	*
***Silene* shoot biomass ~ F2 + R1**						
Nox	0.1263	0.1400	27.00	0.902	0.375	
Nred	0.0258	0.1400	27.00	0.184	0.855	
***Silene* root biomass ~ F2 + R1**						
Nox	0.0163	0.1475	27.00	0.110	0.913	
Nred	0.0527	0.1475	27.00	0.357	0.724	

Biomass was found to be measurably greater with the Nox and Nred additions (compared with the water-only control), but rarely was this significant when data were pooled across soil depth levels. This means that H3.2 (biomass would be greater with nitrogen enrichment) was not clearly supported when data were pooled in this way; biomass response to nitrogen was found to depend on soil depth, and this interaction will be considered in the following section.

3.4.3 Biomass response to interaction of soil depth and nitrogen treatment

3.4.3.1 Community response

Differences in community biomass production were predominantly influenced by soil depth (Figure 3.11, Table 3.11). Community biomass responses were investigated via mixed effect models (Table 3.12).

Soil depth was a significant factor for total ($t = -5.475$, 24 df, $p = 1.25e^{-05}$) and shoot ($t = -7.979$, 20 df, $1.21e^{-07}$) biomass, and near-significant ($t = -2.062$, 24 df, $p = 0.0502$) for root biomass; all biomass metrics were greater in deep soil compared to shallow soil treatments, for all levels of the nitrogen treatment. The biomass response to nitrogen addition varied depending on soil depth - in deep soil, there was no significant difference in the response of total or shoot biomass to nitrogen treatments, whereas in shallow soil, community shoot biomass was significantly greater in the reduced nitrogen treatment compared with the control.

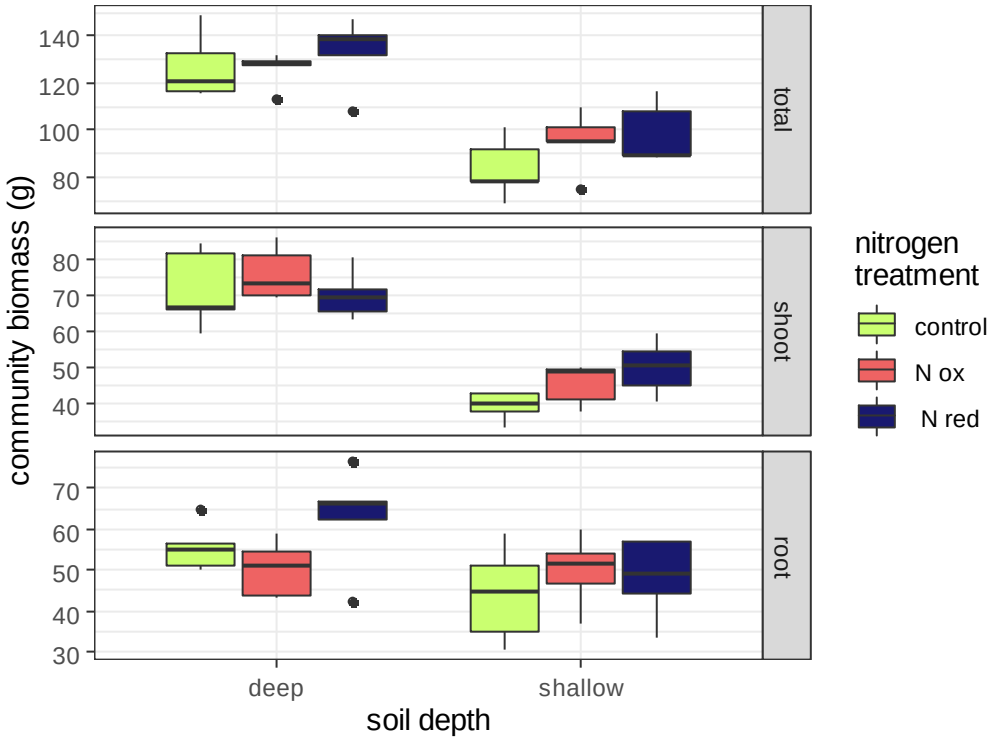


Figure 3.11 Community biomass responses to the interaction of soil depth and nitrogen treatments.

Table 3.11 Mean community biomass in each of the soil depth:nitrogen treatment cases.

		deep soil		shallow soil	
N treatment	**measure**	**mean**	**SD**	**mean**	**SD**
control	total	127.24	14.13	83.62	12.94
	shoot	71.58	10.94	39.54	3.89
	root	55.66	5.81	44.08	11.60
Nox	total	126.20	7.39	95.34	12.78
	shoot	75.96	7.39	45.55	5.53
	root	50.24	6.72	49.80	8.61
Nred	total	132.95	15.12	98.19	13.04
	shoot	70.07	6.68	50.01	7.52
	root	62.89	12.76	48.18	9.85

Table 3.12 Output from mixed effect models for community biomass responses to the interaction of soil depth and nitrogen treatment.

Total community biomass ~ F1 + F2 + F1:F2 + R1						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
shallow	-0.4246	0.0775	24.00	-5.475	$1.25e^{-05}$	***
Nox	-0.0049	0.0775	24.00	-0.064	0.950	
Nred	0.0493	0.0775	24.00	0.554	0.584	
shallow : Nox	0.1378	0.1097	24.00	1.257	0.221	
shallow : Nred	0.1203	0.1097	24.00	1.097	0.284	
Community shoot biomass ~ F1 + F2 + F1:F2 + R1						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
shallow	-0.5883	0.0737	20.00	-7.979	$1.21e^{-07}$	***
Nox	0.0650	0.0737	20.00	0.881	0.389	
Nred	-0.0156	0.0737	20.00	-0.212	0.835	
shallow : Nox	0.0743	0.1043	20.00	0.713	0.484	
shallow : Nred	0.2453	0.1043	20.00	2.353	0.029	*
Community root biomass ~ F1 + F2 + F1:F2 + R1						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
shallow	-0.2579	0.1250	24.00	-2.062	0.0502	^
Nox	-0.1054	0.1250	24.00	-0.843	0.4074	
Nred	0.1072	0.1250	24.00	0.857	0.3999	
shallow : Nox	0.2432	0.1768	24.00	1.375	0.1818	
shallow : Nred	-0.0080	0.1768	24.00	-0.045	0.9645	

3.4.3.2 Species response

Species' responses to nitrogen addition varied, depending on soil depth (Table 3.13, Figure 3.12, Figure 3.13).

Table 3.13 Mean biomass (g) across all treatment combinations. In each case, n = 5. "Shoot" refers to above-ground biomass;"root" refers to below-ground biomass; "shallow" and "deep" refer to soil depth treatments; nitrogen treatments are denoted as follows: "control" = control group; "Nox" = oxidised nitrogen ($NaNO_3$) group; "Nred" = reduced nitrogen group (NH_4Cl). Data are presented as mean ± standard deviation.

Nitrogen treatment	Biomass measure	*Lotus corniculatus*		*Dactylis glomerata*		*Silene vulgaris*	
		deep	shallow	deep	shallow	deep	shallow
Control	total	65.7 ± 11.4	51.7 ± 11.4	30.8 ± 4.4	12.5 ± 2.1	30.8 ± 5.4	19.4 ± 1.5
	shoot	41.7 ± 8.0	26.4 ± 4.3	21.3 ± 3.3	7.0 ± 1.3	8.6 ± 4.3	6.1 ± 1.0
	root	24.0 ± 5.9	25.3 ± 8.5	9.5 ± 1.1	4.9 ± 2.0	22.2 ± 1.5	13.3 ± 1.6
Nox	total	60.1 ± 6.6	51.4 ± 6.5	36.5 ± 5.6	21.2 ± 5.2	29.6 ± 5.7	22.7 ± 6.4
	shoot	40.5 ± 4.0	24.9 ± 3.4	26.6 ± 4.2	13.1 ± 1.9	8.9 ± 2.4	7.5 ± 2.5
	root	19.6 ± 6.0	26.5 ± 4.4	9.9 ± 1.6	8.13 ± 3.4	20.8 ± 3.7	15.2 ± 4.1
Nred	total	64.9 ± 11.3	54.2 ± 9.9	34.3 ± 9.3	24.3 ± 6.6	33.8 ± 4.8	19.7 ± 4.8
	shoot	38.9 ± 4.6	29.4 ± 5.3	22.8 ± 5.6	14.2 ± 4.4	8.3 ± 1.3	6.4 ± 1.9
	root	26.0 ± 7.1	24.8 ± 6.3	11.5 ± 4.3	10.1 ± 3.7	25.4 ± 5.5	13.3 ± 3.2

Species' total and shoot biomass metrics were consistently greater in the deep soil treatment, for all nitrogen treatment levels (Figure 3.12). Root biomass response varied between species, with both *Dactylis* and *Silene* root biomass being greater in deep soil, across all nitrogen treatments. *Lotus* root biomass bucked this trend, however, and was greater in deep soils in the reduced nitrogen treatment, but not in the shallow soils in the control and oxidised nitrogen groups.

Lotus total biomass was lower in the oxidised nitrogen group (mean 55.7 g across both soil depth treatments) compared to the reduced nitrogen (mean 59.5 g) and control (mean 58.7 g) groups. *Lotus* total and shoot biomass was reduced across both nitrogen treatments in deep

soil. A lower mean shoot biomass in the oxidised nitrogen treatment resulted in a lower total biomass for both soil depth groups, despite an increase in root biomass in the oxidised nitrogen group. In contrast, reduced nitrogen additions to shallow soil mesocosms saw an increase in both shoot and total *Lotus* biomass.

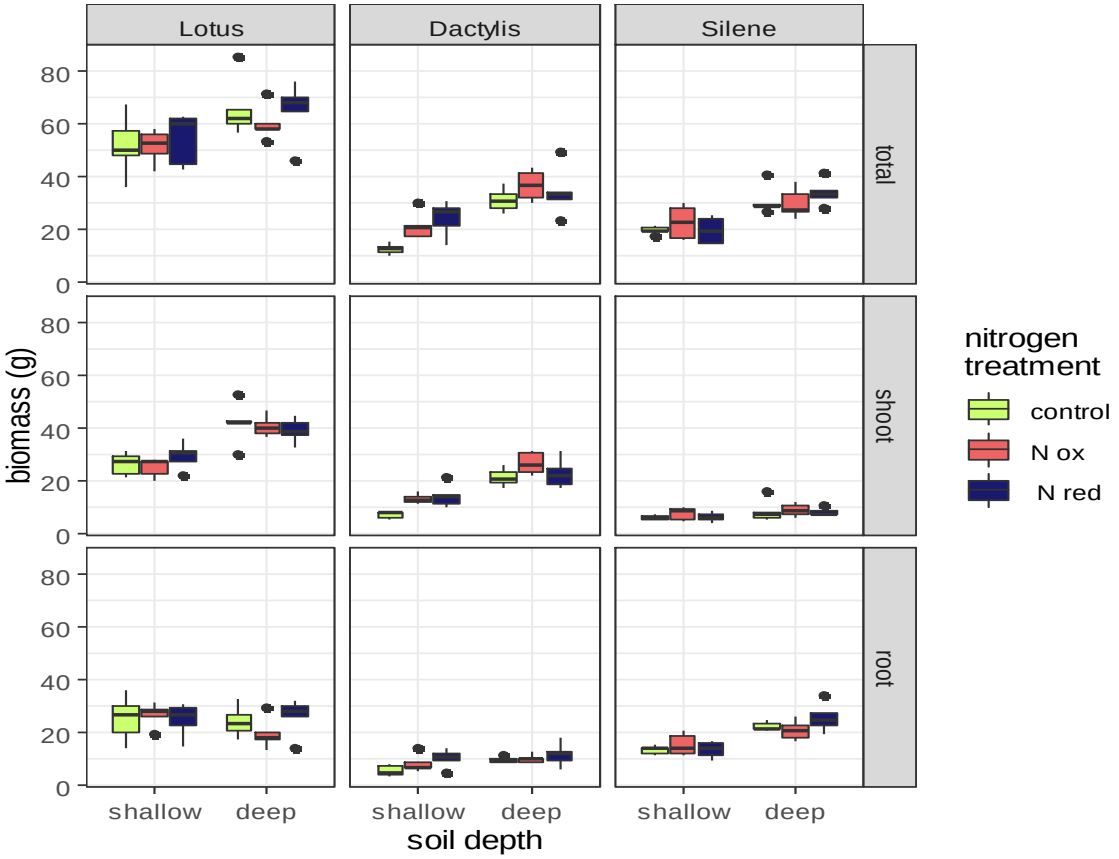


Figure 3.12 Species' biomass responses to interaction of soil depth and nitrogen treatment.

The increase in *Dactylis* total biomass under nitrogen-addition treatments was only significant for *Dactylis* growing in shallow soil and was driven by greater shoot *Dactylis* biomass; the root biomass in these cases was also greater in the nitrogen-addition group compared to the control group, but not significantly so.

Silene consistently showed an increase in all biomass measures with nitrogen addition in shallow soil mesocosms, albeit not significantly; an increase in shoot biomass in the oxidised nitrogen treatment did not fully balance a corresponding reduction in root biomass, leading to an overall mean reduction in total biomass in the oxidised nitrogen treatment in deep soil mesocosms, compared to the water-only control group.

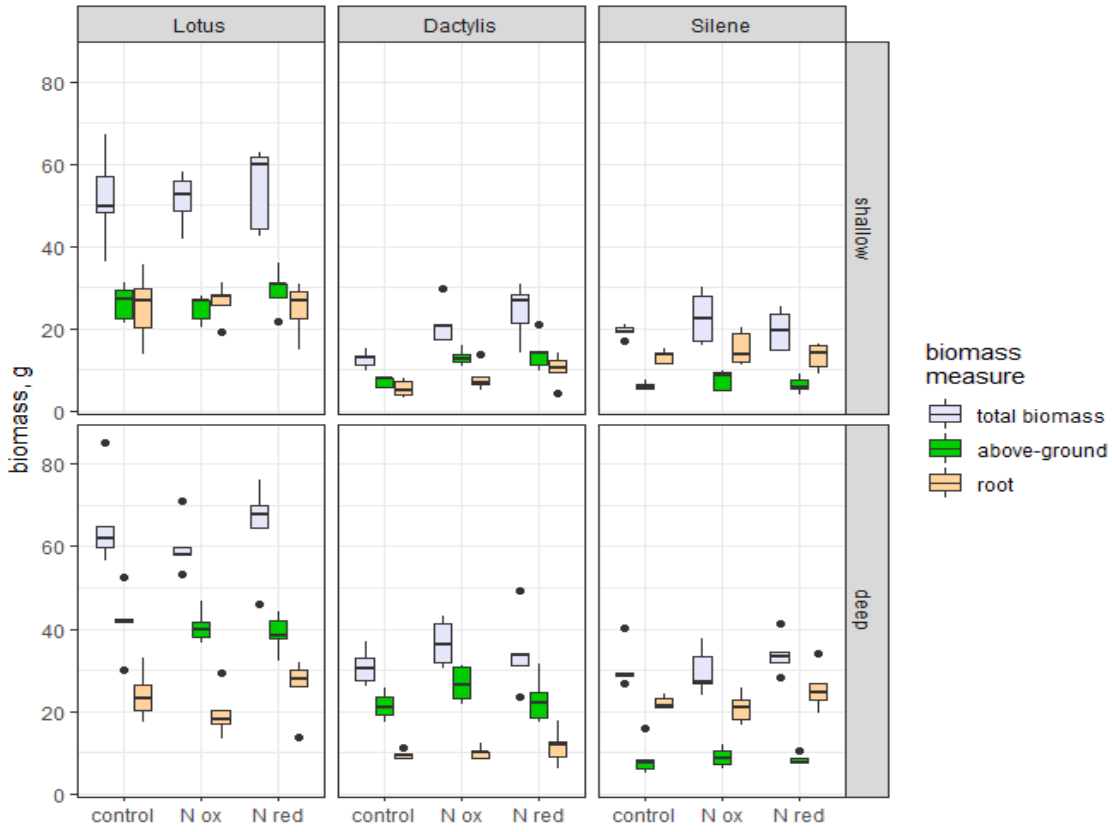


Figure 3.13 Species' biomass response across nitrogen treatments in each soil depth treatment.

The nature of individual species' biomass response to interaction between soil depth and nitrogen treatment was investigated via linear mixed effect models. Predicted values for all species' shoot and root biomass are illustrated below in Figure 3.14. Mixed effect model coefficients and pairwise comparisons (i.e. values averaged across all levels of the alternative factor) are appended below in Table 3.14 (*Lotus*), Table 3.15 (*Dactylis*), and Table 3.16 (*Silene*).

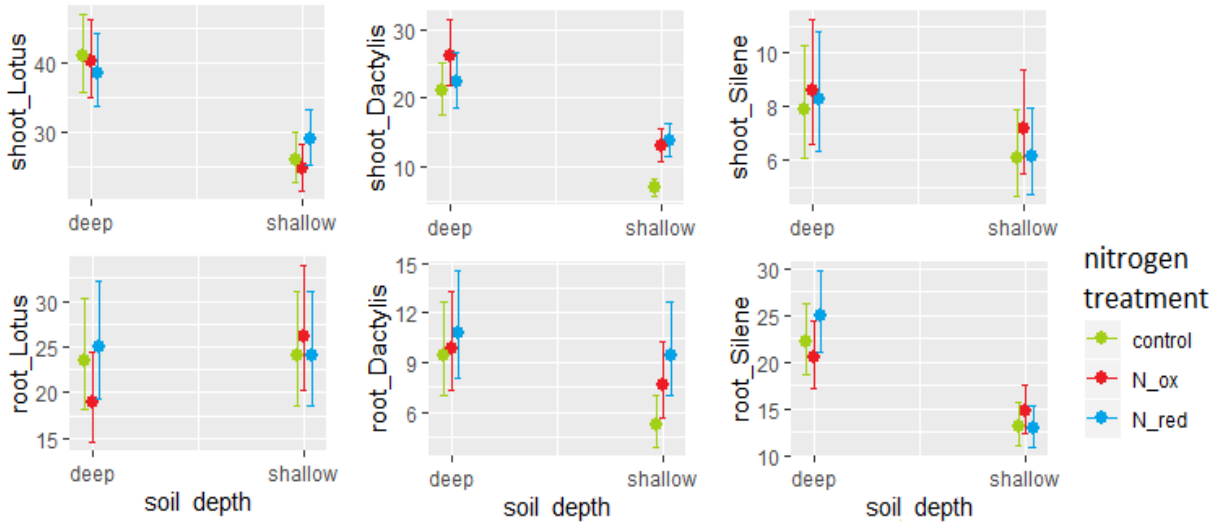


Figure 3.14 Predicted values for species' shoot and root biomass responses to interaction between soil depth and nitrogen treatment.

Table 3.14 Lotus biomass responses – output from mixed effects model.

model						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
***Lotus* total biomass ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.2495	0.1097	24.00	-2.275	0.0321	*
Nox	-0.0832	0.1097	24.00	-0.758	0.4556	
Nred	-0.0157	0.1097	24.00	-0.143	0.8876	
shallow : Nox	0.0916	0.1551	24.00	0.591	0.5603	
shallow : Nred	0.0698	0.1551	24.00	0.450	0.6568	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	0.196	0.0633	20	3.091	0.0058	
control – Nox	0.037	0.0775	20	0.482	0.8806	
control – Nred	-0.019	0.0775	20	-0.248	0.9668	
Nox – Nred	-0.057	0.0775	20	-0.730	0.7490	
***Lotus* shoot biomass ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.4515	0.0992	24.00	-4.554	0.0001	***
Nox	-0.0161	0.0992	24.00	-0.162	0.8728	
Nred	-0.0589	0.0992	24.00	-0.594	0.5581	
shallow : Nox	-0.0395	0.1402	24.00	-0.282	0.7805	
shallow : Nred	0.1648	0.1402	24.00	1.175	0.2513	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	0.41	0.0572	20	7.157	<0.0001	
control – Nox	3.49	0.0496	20	3.38	3.59	
control – Nred	3.45	0.0496	20	3.35	3.56	
Nox – Nred	3.51	0.0496	20	3.41	3.62	

Table 3.14 continued over ...

... Table 3.14 continued

***Lotus* root biomass ~ F1 + F2 + F1:F2 + R1**						
shallow	0.0215	0.1744	20.00	0.123	0.903	
Nox	-0.2179	0.1744	20.00	-1.250	0.226	
Nred	0.0610	0.1744	20.00	0.350	0.730	
shallow : Nox	0.3054	0.2466	20.00	1.239	0.230	
shallow : Nred	-0.0592	0.2466	20.00	-0.240	0.813	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	-0.104	0.1010	20	-1.029	0.3157	
control – Nox	0.065	0.123	20	0.529	0.8583	
control – Nred	-0.031	0.123	20	-0.254	0.9650	
Nox – Nred	-0.097	0.123	20	-0.783	0.7175	

Table 3.15 Dactylis biomass responses - model coefficients and pairwise comparisons of levels of main factors.

model						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
***Dactylis* total biomass ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.9032	0.1230	20.00	-7.343	$4.27e^{-07}$	***
Nox	0.1683	0.1230	20.00	1.369	0.1863	
Nred	0.0876	0.1230	20.00	0.712	0.4845	
shallow : Nox	0.3500	0.1739	20.00	2.012	0.0579	^
shallow : Nred	0.5515	0.1739	20.00	3.170	0.0048	**
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	0.603	0.071	20	8.487	<0.0001	
control – Nox	-0.343	0.087	20	-3.948	0.0022	
control – Nred	-0.363	0.087	20	-4.178	0.0013	
Nox – Nred	-0.020	0.087	20	-0.230	0.9712	

Table 3.15 continued over ...

...Table 3.15 continued

***Dactylis* shoot biomass ~ F1 + F2 + F1:F2 + R1**						
shallow	-1.1191	0.1008	20.00	-11.106	$5.28e^{-10}$	***
Nox	0.2190	0.1008	20.00	2.174	0.0419	*
Nred	0.0551	0.1008	20.00	0.546	0.5908	
shallow : Nox	0.4150	0.1425	20.00	2.912	0.0086	**
shallow : Nred	0.6321	0.1425	20.00	4.436	0.0003	***

Model pairwise comparisons					
comparison	estimate	SE	df	t-ratio	p
deep – shallow	0.770	0.0582	20	13.236	<0.0001
control – Nox	-0.427	0.0713	20	-5.986	<0.0001
control – Nred	-0.371	0.0713	20	-5.209	0.0001
Nox – Nred	0.055	0.0713	20	0.778	0.7207

***Dactylis* root biomass ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.5862	0.2050	20.00	-2.863	0.0096	**
Nox	0.0440	0.2050	20.00	0.215	0.8323	
Nred	0.1349	0.2050	20.00	0.658	0.5178	
shallow : Nox	0.3348	0.2899	20.00	1.155	0.2617	
shallow : Nred	0.4477	0.2899	20.00	1.544	0.1382	

Model pairwise comparisons					
comparison	estimate	SE	df	t-ratio	p
deep – shallow	0.326	0.118	20	2.754	0.0122
control – Nox	-0.211	0.145	20	-1.458	0.3314
control – Nred	-0.359	0.145	20	-2.475	0.0558
Nox – Nred	-0.147	0.145	20	-1.017	0.5750

Table 3.16 Silene biomass responses - model coefficients and pairwise comparisons of levels of main factors.

model						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
***Silene* total biomass ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.4511	0.1241	24.00	-3.634	0.0013	**
Nox	-0.0402	0.1241	24.00	-0.324	0.7489	
Nred	0.0974	0.1241	24.00	0.784	0.4405	
shallow : Nox	0.1654	0.1755	24.00	0.942	0.3554	
shallow : Nred	-0.1063	0.1755	24.00	-0.605	0.5506	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	0.431	0.0717	20	6.019	<0.0001	
control – Nox	-0.043	0.0878	20	-0.484	0.8795	
control – Nred	-0.044	0.0878	20	-0.504	0.8703	
Nox – Nred	-0.002	0.0878	20	-0.019	0.9998	
***Silene* shoot biomass ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.2640	0.1899	24.00	-1.386	0.178	
Nox	0.0864	0.1899	24.00	0.455	0.653	
Nred	0.0439	0.1899	24.00	0.231	0.819	
shallow : Nox	0.0798	0.2686	24.00	0.297	0.769	
shallow : Nred	-0.0363	0.2686	24.00	-0.135	0.894	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	0.249	0.11	20	2.269	0.0345	
control – Nox	-0.126	0.134	20	-0.941	0.6217	
control – Nred	-0.026	0.134	20	-0.192	0.9799	
Nox – Nred	0.101	0.134	20	0.749	0.7379	

Table 3.16 continued over ...

...Table 3.16 continued

***Silene* root biomass ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.5154	0.1217	20.00	-4.234	0.0004	***
Nox	-0.0761	0.1217	20.00	-0.625	0.5391	
Nred	0.1227	0.1217	20.00	1.008	0.3253	
shallow : Nox	0.1847	0.1721	20.00	1.073	0.2961	
shallow : Nred	-0.1401	0.1721	20.00	-0.814	0.4252	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	0.501	0.0703	20	7.123	<0.0001	
control – Nox	-0.016	0.0861	20	-0.189	0.9805	
control – Nred	-0.053	0.0861	20	-0.612	0.8152	
Nox – Nred	-0.036	0.0861	20	-0.423	0.9066	

The relative direction and magnitude of biomass response to nitrogen addition compared with the control level was assessed for each of the two soil depths (Table 3.17).

Table 3.17 Indication of direction of biomass change (reference level = control), and nitrogen-addition treatment with the greater magnitude of change (Nox, Nred). + indicates greater biomass, - indicates less biomass.

		deep soil			**shallow soil**		
		direction of change		**greater magnitude**	**direction of change**		**greater magnitude**
level	**biomass**	**Nox**	**Nred**		**Nox**	**Nred**	
community	total	-	+	Nred	+	+	Nred
	shoot	+	-	Nox	+	+	Nred
	root	-	+	Nred	+	+	Nox
species							
Lotus	total	-	-	Nox	+	+	Nred
	shoot	-	-	Nox	-	+	Nred
	root	-	+	Nred	+	+	Nred
Dactylis	total	+	+	Nox	+	+	Nred
	shoot	+	+	Nox	+	+	Nred
	root	+	+	Nred	+	+	Nred
Silene	total	-	+	Nred	+	-	Nox
	shoot	+	+	Nox	+	+	Nox
	root	-	+	Nred	-	+	Nox

It can be seen that the data do not fully support H3.3 (that biomass will have a stronger, positive response to Nox compared to Nred). Not only is there no consistently positive response to nitrogen addition, for either Nox or Nred, but also individual species' responses vary depending on nitrogen form and soil depth treatment combinations. In deep soil, oxidised and reduced nitrogen treatments each account for six occasions of greatest difference from the mean biomass in the control group. In shallow soil, the reduced nitrogen treatment is associated with eight cases of greatest magnitude of change, compared to four cases for the oxidised nitrogen treatment. This illustrates the importance of species identity in trying to predict direction and magnitude of biomass changes related to changing availability of nitrogen and soil depth.

3.4.4 Root:shoot (R:S) ratio

Associated hypothesis:

- H3.4 The root:shoot ratio will decrease a) in deeper soil treatments, and b) with nitrogen addition.

Allocation of resources was investigated via the root:shoot ratio (Table 3.18, Figure 3.15): in response to nutritional or water stress, plants will tend to divert resources into root biomass at the expense of shoot tissue, resulting in a higher root:shoot ratio in such circumstances. Mixed effect models indicated that all species' root:shoot ratios were most strongly influenced by soil depth (Table 3.19); no species showed a significant response to nitrogen treatment, or the interaction between soil depth and nitrogen addition.

The root:shoot ratios for *Lotus* and *Dactylis* were significantly higher in the shallow soil treatment compared to the deep soil treatment, indicating a proportionately greater allocation of biomass to root in those species. This suggested that *Lotus* and *Dactylis* were experiencing nutritional or drought-related stress in the shallow soil mesocosms. *Silene* root:shoot ratio did not respond significantly to either soil depth or nitrogen addition, or to their interaction.

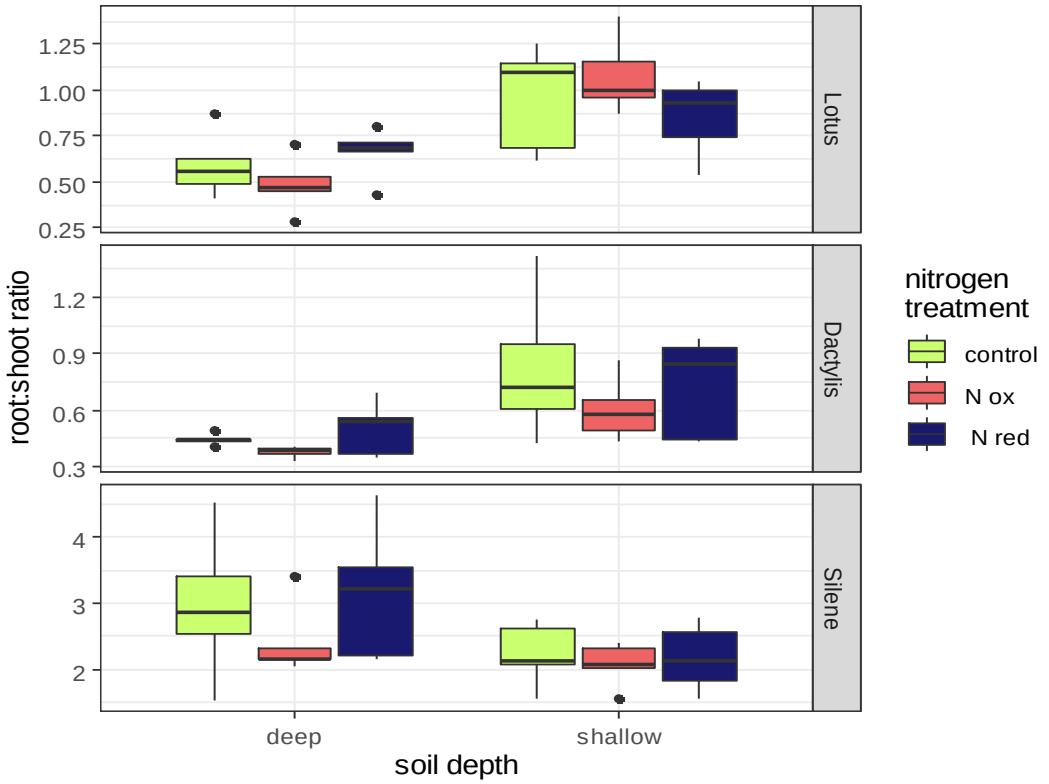


Figure 3.15 Root:shoot ratio responses to the interaction of soil depth and nitrogen treatments.

Table 3.18 Mean root:shoot ratio for each species across all treatment combinations.

	DEEP SOIL			**SHALLOW SOIL**		
N treatment	control	N ox	N red	control	N ox	N red
Lotus corniculatus	0.59	0.49	0.66	0.96	1.08	0.85
Dactylis glomerata	0.45	0.38	0.50	0.83	0.61	0.73
Silene vulgaris	2.97	2.42	3.16	2.22	2.07	2.17

Lotus and *Dactylis* mean R:S ratios were greatest in shallow soil mesocosms; *Silene* R:S mean ratio was greatest in the deep soil treatment. *Silene* R:S ratios significantly exceeded those of both *Dactylis* and *Lotus*, in all treatment combinations, due to the thick, starchy roots produced by this species.

In deep soil, all three species had a greater R:S ratio in the reduced nitrogen treatment and a lower R:S ratio in the oxidised nitrogen group, compared to the water-only control. In shallow soil, nitrogen addition led to reduced relative root growth in both *Dactylis* and *Silene* (i.e. they had lower R:S ratio). Nitrogen addition in the shallow soil group promoted an increase in relative *Lotus* root biomass under the oxidised nitrogen addition, and a decrease in root biomass under the reduced nitrogen addition.

Table 3.19 Model outputs for all species' root:shoot ratio responses to soil depth and nitrogen treatments.

model						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
***Lotus* root:shoot ratio ~ F1 + F2 + F1:F2 + R1**						
shallow	0.4730	0.1540	20	3.071	0.0060	**
Nox	-0.2018	0.1540	20	-1.310	0.2049	
Nred	0.1199	0.1540	20	0.778	0.4455	
shallow : Nox	0.3450	0.2178	20	1.584	0.1290	
shallow : Nred	-0.2241	0.2178	20	-1.029	0.3159	
***Dactylis* root:shoot ratio ~ F1 + F2 + F1:F2 + R1**						
shallow	0.5324	0.1774	20	3.001	0.0071	**
Nox	-0.1751	0.1774	20	-0.987	0.3355	
Nred	0.0799	0.1774	20	0.450	0.6574	
shallow : Nox	-0.0803	0.2509	20	-0.320	0.7524	
shallow : Nred	-0.1845	0.2509	20	-0.735	0.4707	
***Silene* root:shoot ratio ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.2521	0.1568	20	-1.608	0.124	
Nox	-0.1625	0.1568	20	-1.036	0.312	
Nred	0.0788	0.1568	20	0.503	0.621	
shallow : Nox	0.1049	0.2217	20	0.473	0.641	
shallow : Nred	-0.1039	0.2217	20	-0.468	0.645	

The data partially supported both parts of Hypothesis H3.4. Deeper soil resulted in a reduced root:shoot ratio for *Lotus* and *Dactylis* in all nitrogen treatments; *Silene*, however, had greater root:shoot ratio in shallow soil compared with deep soil, across all nitrogen treatments. Observed root:shoot responses to nitrogen addition were less predictable, depending on species and soil depth. All species had a lower root:shoot ratio in Nox treatment in deep soil, and in Nred in shallow soil; similarly, all species had a greater root:shoot ratio than that of the control in Nred in deep soil. *Lotus* root:shoot ratio in Nox in shallow soil was greater than that of the control, whereas *Dactylis* and *Silene* both had lower observed root:shoot ratios in this treatment combination.

3.4.5 Aerial cover

A general pattern of decline in aerial cover was noted from south to north along the array (i.e. from Block 1 to Block 5) (Figure 3.16).

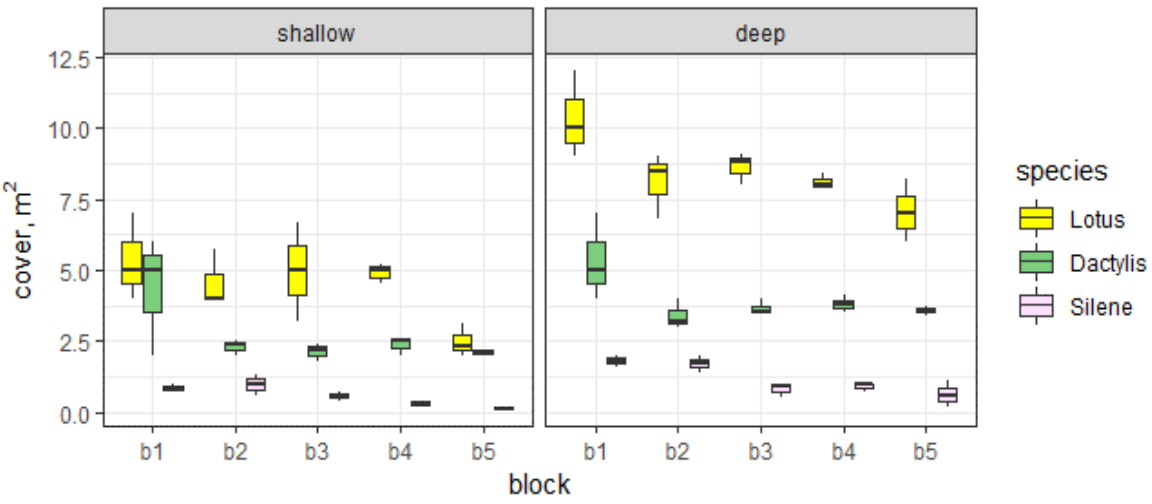


Figure 3.16 Change in species' cover along experimental array. Block 1 (b1) is to the south (n = 30).

Wilcoxon pairwise comparisons indicated that changes in *Lotus* aerial cover between Block 1 and Block 5 were significant in mesocosms with deep soils ($p < 0.05$), though not in those with shallow soils at $p<0.05$ level. *Dactylis* cover, though measurable, was not significantly different between Block 1 and Block 5 at $p<0.05$ level; cover levels in Block 1 were found to be atypically higher than those in the other blocks. *Silene* cover showed a significant difference between cover values from one end of the array to the other ($\chi^2 = 14.108$, 4 df, $p < 0.01$). Cover values in Block 1 and Block 2 were higher than those in Blocks 3-5, in both soil depth treatments.

Linear mixed effect models, with block as a random effect, indicated that differences in species' cover were driven primarily by soil depth – for all species, mean cover was less in shallow soil mesocosms across all nitrogen treatments (Table 3.20, Figure 3.17), being between 38% and 79% of that in the deep soil treatment. Over all treatments, *Lotus* produced the most extensive cover (mean 0.065 m^2), with lower values for *Dactylis* (mean 0.033 m^2) and *Silene* (mean 0.009 m^2) cover reflecting their more upright growth habit.

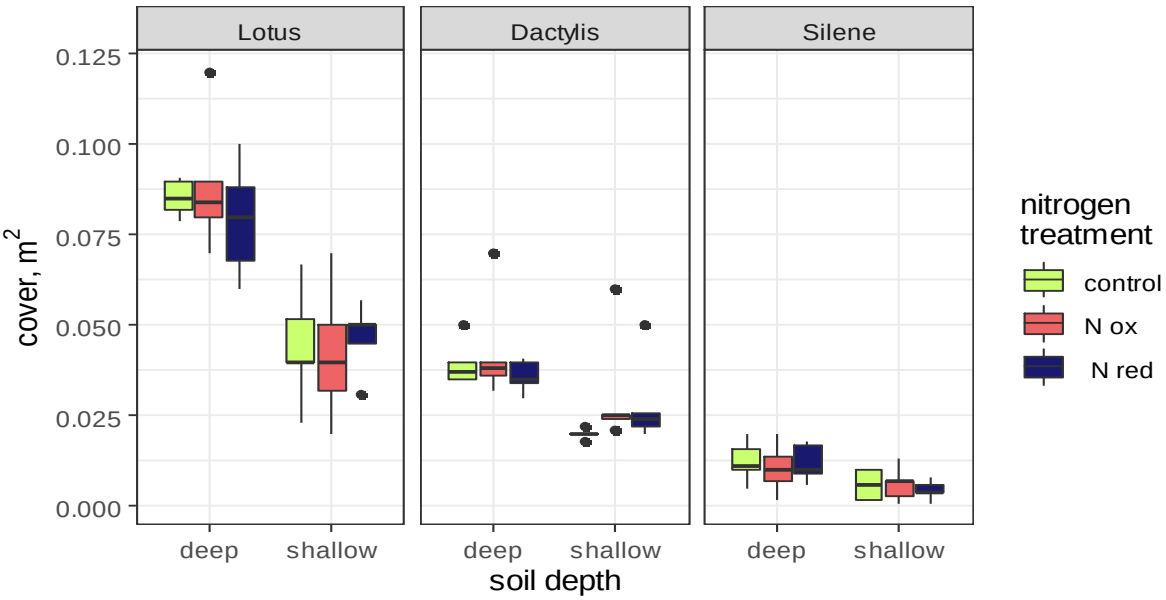


Figure 3.17 Species' cover response to interaction of soil depth and nitrogen treatments.

Table 3.20 Mean aerial cover across soil depth and nitrogen treatments (m^2).

	DEEP SOIL			**SHALLOW SOIL**		
N treatment	control	Nox	Nred	control	Nox	Nred
Lotus corniculatus	0.0854	0.0888	0.0792	0.0444	0.0424	0.0466
Dactylis glomerata	0.0394	0.0432	0.0360	0.0200	0.0310	0.0284
Silene vulgaris	0.0124	0.0106	0.0120	0.0060	0.0062	0.0046

Linear mixed effect models indicated that species responded differently to soil depth and nitrogen treatment, and their interaction (Table 3.21, Figure 3.18).

Table 3.21 Model coefficients from linear mixed effect models, for species cover responses to soil depth and nitrogen treatments.

model						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
***Lotus* cover ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.7121	0.1391	20	-5.121	$5.21e^{-05}$	***
Nox	0.0238	0.1391	20	0.171	0.866	
Nred	-0.0902	0.1391	20	-0.649	0.524	
shallow : Nox	-0.0955	0.1967	20	-0.486	0.633	
shallow : Nred	0.1779	0.1967	20	0.905	0.376	

Table 3.21 continued over ...

... Table 3.21 continued

***Dactylis* cover ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.6708	0.1180	20	-5.683	$1.46e^{-05}$	***
Nox	0.0603	0.1180	20	0.511	0.6148	
Nred	-0.0874	0.1180	20	-0.741	0.4674	
shallow : Nox	0.2969	0.1669	20	1.779	0.0905	^
shallow : Nred	0.3807	0.1669	20	2.281	0.0337	*
***Silene* cover ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.8590	0.2715	20	-3.164	0.0049	**
Nox	-0.3004	0.2715	20	-1.106	0.2817	
Nred	-0.0126	0.2715	20	-0.046	0.9634	
shallow : Nox	0.2548	0.3839	20	0.664	0.5145	
shallow : Nred	-0.2153	0.3839	20	-0.561	0.5812	

In deeper soil, *Lotus* cover in the oxidised nitrogen treatment was greater than that in the control in deep soil, and less than that in control with the reduced nitrogen treatment. This pattern was reversed in the shallow soil treatment. The difference in response between both nitrogen addition treatments and the control group was not significant in either deep or shallow soil treatments.

In deep soil, *Silene* cover was inhibited by both oxidised and reduced nitrogen, compared to the control; the negative response was greater under Nox than under Nred, which was equivalent to the control cover value. In shallow soil, this pattern changed, with cover being slightly (non-significantly) greater under the Nox treatment, and reduced under the Nred treatment, compared to the control. The scale of *Silene* cover response to the soil and nitrogen addition treatment interaction was also not significant, between the two soil depth groups.

Dactylis cover response to nitrogen treatment varied significantly, depending on soil depth (Figure 3.18). In deep soil, cover was slightly greater with the oxidised nitrogen treatment, and reduced with the reduced nitrogen treatment; in shallow soil, this response changed, and both nitrogen addition treatments resulted in greater cover values. The cover response to the interaction of soil depth and nitrogen addition was significantly different between the two soil depth groups for the reduced nitrogen treatment ($t = 2.281$, 20 df, $p = 0.0337$), and near-significant for the oxidised nitrogen treatment ($t = 1.779$, 20 df, $p = 0.0905$).

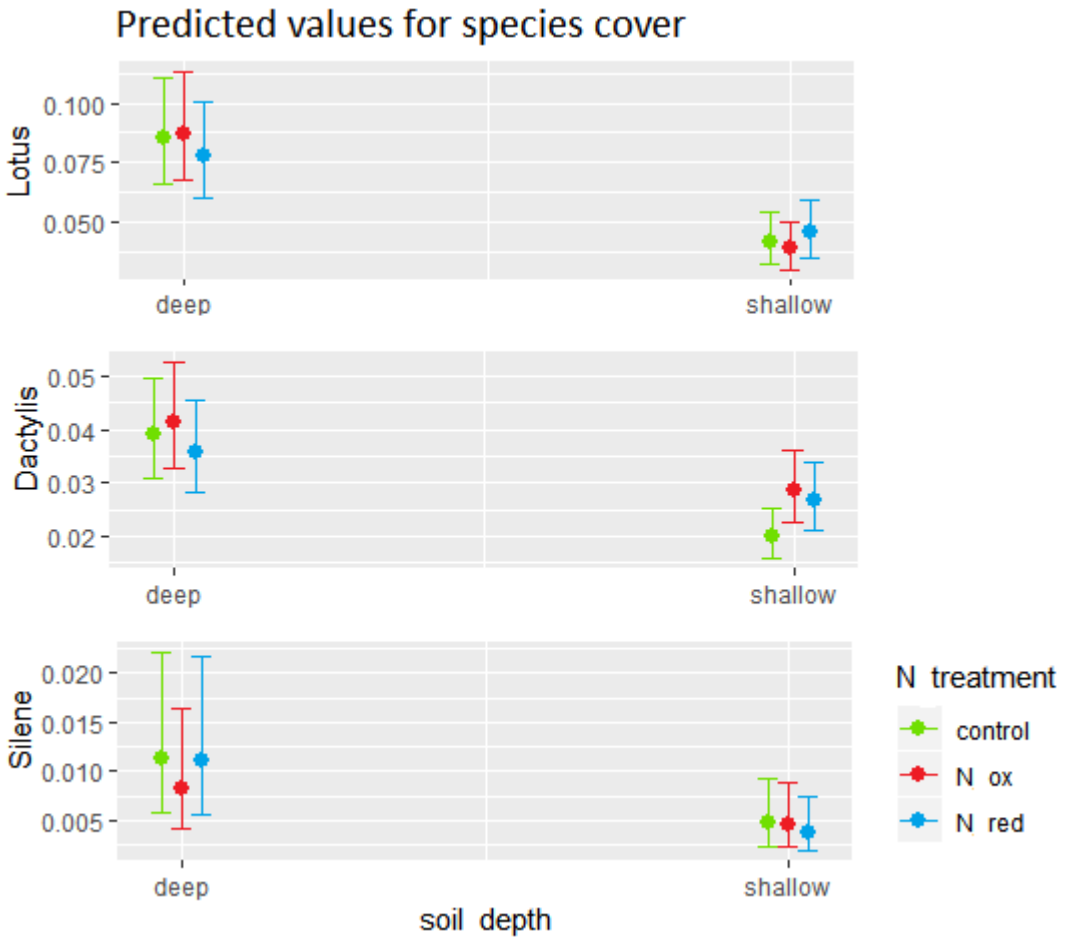


Figure 3.18 Marginal effects of interaction terms in species' cover models (cover ~ soil depth + N treatment + soil depth:N treatment). Error bars are SE.

3.4.6 Flowering effort

Silene and *Dactylis* flower counts were significantly higher in deeper soil (Figure 3.19, Table 3.22); *Lotus* flowering effort showed no response to soil depth.

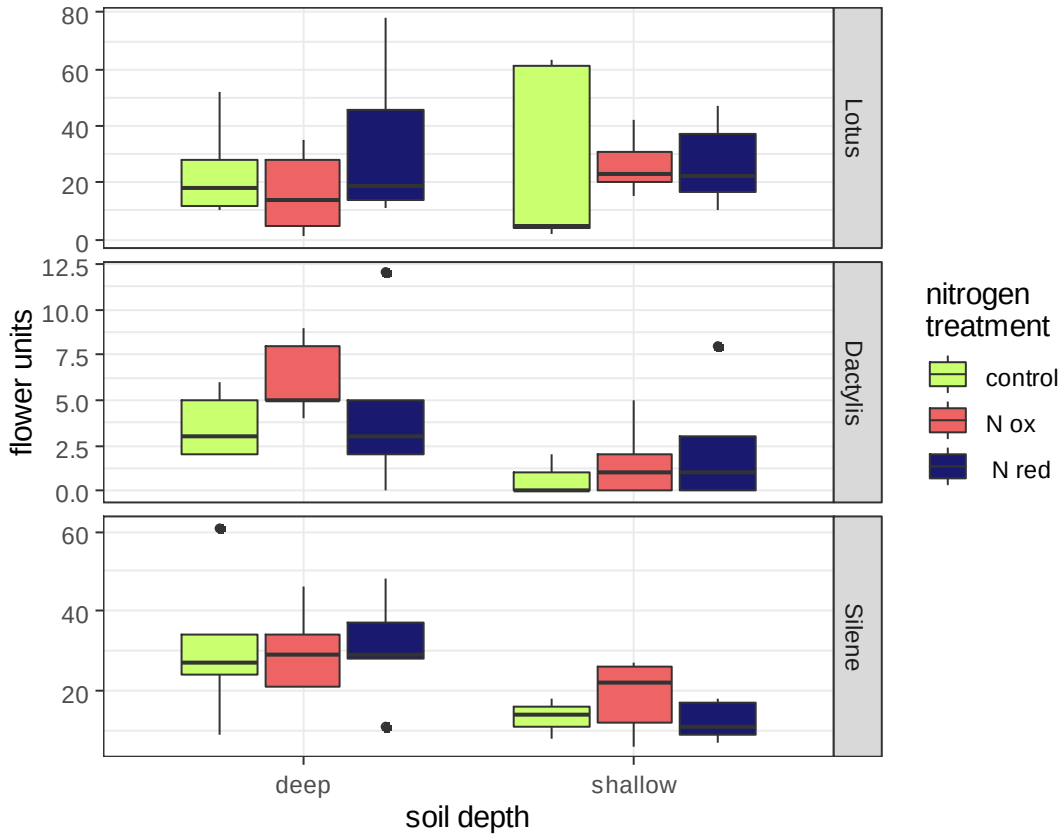


Figure 3.19 Species' flowering effort (flower units) across all treatment combinations (n = 30).

Table 3.22 Mean flower unit counts across all soil depth and nitrogen treatment combinations. For each case, n = 5.

	CONTROL		N_{OX}		N_{RED}	
Species	**deep**	**shallow**	**deep**	**shallow**	**deep**	**shallow**
Lotus corniculatus	24.0	27.0	16.6	26.2	33.6	26.6
Dactylis glomerata	3.6	0.6	6.2	1.6	4.4	2.4
Silene vulgaris	31.0	13.4	30.2	18.6	30.6	12.4

Mixed effect models indicated that soil depth was a significant factor in flowering effort for *Dactylis* and *Silene*, but not for Lotus (Table 3.23Table 1.1). Nitrogen treatment did not significantly influence flowering effort in any species, across both soil depth treatments, although there was a measurable increase in *Dactylis* flowering effort with nitrogen addition in both deep and shallow soils, compared with the control.

Table 3.23 Model output and significant pairwise comparisons for all species' flowering effort.

model						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
***Lotus* flowers ~ F1 + F2 + F1:F2 + R1**						
shallow	3.000	13.319	20	0.225	0.824	
Nox	-7.400	13.319	20	-0.556	0.585	
Nred	9.600	13.319	20	0.721	0.479	
shallow : Nox	6.600	18.836	20	0.350	0.730	
shallow : Nred	-10.000	18.836	20	-0.531	0.601	
***Dactylis* flower stems ~ F1 + F2 + F1:F2 + R1**						
shallow	-3.00	1.625	20	-1.846	0.0797	^
Nox	2.600	1.625	20	1.600	0.1252	
Nred	0.800	1.625	20	0.492	0.6278	
shallow : Nox	-1.600	2.298	20	-0.696	0.4943	
shallow : Nred	1.000	2.298	20	0.435	0.6681	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	3.2	0.938	20	3.411	0.0028	
***Silene* flowers ~ F1 + F2 + F1:F2 + R1**						
shallow	-17.600	7.220	24	-2.438	0.0226	*
Nox	-0.800	7.220	24	-0.111	0.9127	
Nred	-0.400	7.220	24	-0.055	0.9563	
shallow : Nox	6.000	10.210	24	0.588	0.5623	
shallow : Nred	-0.600	10.210	24	-0.059	0.9536	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	15.8	4.17	20	3.790	0.0011	

Flowering effort showed a strong positive correlation with shoot biomass for *Dactylis* and *Silene* in both soil depth groups, and a strong negative relationship with *Lotus* root biomass in shallow soil (Table 3.24).

Table 3.24 Pearson's correlation coefficient table for flower unit counts with productivity and tissue nutrient content measures for both levels in the soil depth treatment. Significance levels are indicated as per Table 3.3. For all cases, n = 15.

	DEEP SOIL			SHALLOW SOIL		
measure	***Lotus***	***Dactylis***	***Silene***	***Lotus***	***Dactylis***	***Silene***
shoot biomass	-0.28	0.60 *	0.74	0.11	0.74 **	0.62 *
root biomass	0.20	0.11	0.35	-0.62 *	0.44	0.62 *
shoot tissue N	0.10	0.59 *	0.25	-0.78 ***	0.47	0.23
shoot tissue C	0.08	0.35	0.08	0.05	-0.03	0.25
root tissue N	-0.38	0.29	0.52 *	-0.10	-0.42	-0.23
root tissue C	-0.05	0.04	-0.07	-0.09	0.02	-0.33
cover	-0.19	0.39	-0.16	-0.48	-0.04	0.58 *

Block was found to be a significant factor in *Lotus* flowering effort in shallow soil ($\chi^2 = 9.575$, 4 df, $p < 0.05$) but not in mesocosms with deep soil, or for either *Dactylis* or *Silene*. Overall flower counts in bock 1 were found to be lower than the other blocks, though *Silene* flowering effort in shallow soils showed a negative trend northwards along the array, towards Block 5 (Figure 3.20). *Dactylis* showed no discernible pattern with regard to block.

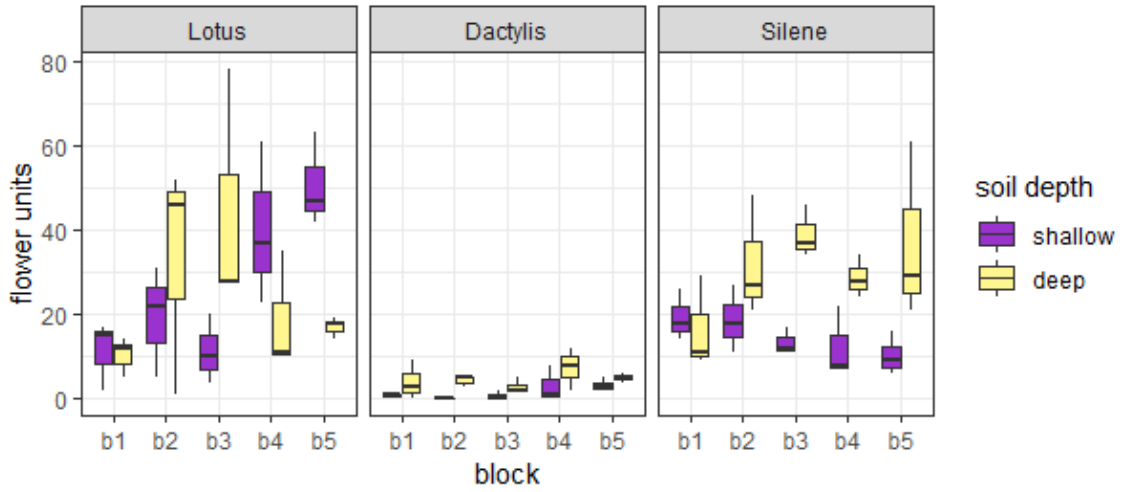


Figure 3.20 Block as a factor in flowering effort. Block 1 (b1) is towards the south.

3.4.7 Tissue carbon and nitrogen

Associated hypothesis:

- H3.5 Tissue nitrogen content will be greater in nitrogen-enriched conditions.

The response of tissue N varied by species, and depended on the form of nitrogen and also the soil depth involved. Actual tissue nitrogen content is reported in section 3.4.7.1; tissue C:N ratio was also investigated, and is reported in section 3.4.7.2. Both have relevance for this hypothesis.

3.4.7.1 Post-experiment plant tissue nitrogen and carbon content

As expected, tissue nitrogen content was consistently higher in shoot material than in root material (Figure 3.21, Table 3.25). *Lotus* had the highest overall tissue nitrogen content of all three species, in both shoot and root tissues. Mean shoot tissue nitrogen content was lower under the Nred treatment in all species growing in deep soil treatment mesocosms, compared with the relevant control. Shoot tissue nitrogen was higher under Nox in *Lotus* and *Dactylis* in the deep soil treatment; in shallow soil, Lotus shoot tissue nitrogen was reduced in both nitrogen addition treatments compared to the control, and *Dactylis* shoot tissue nitrogen was higher under both nitrogen addition treatments. *Silene* shoot tissue nitrogen was lower under both nitrogen additions in the deep soil treatment, compared with the control.

In shallow soil, all three species' root tissue nitrogen content was reduced in both nitrogen-addition treatments compared with the shallow soil control; this was also the case for *Dactylis* in deep soil. *Lotus* root tissue nitrogen content in deep soil followed the same pattern as shoot tissue, i.e. higher under Nox and lower under Nred; *Silene* root tissue nitrogen did not change in the deep soil mesocosms, regardless of nitrogen addition.

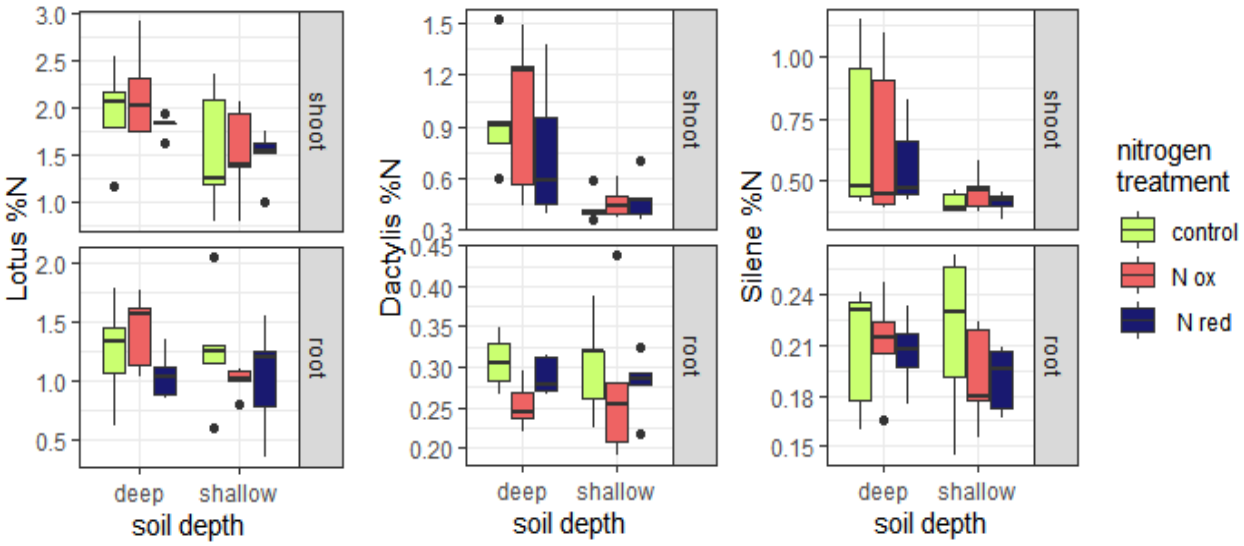


Figure 3.21 Percent plant tissue nitrogen content.

Table 3.25 Mean percentage shoot and root tissue nitrogen content across all soil and nitrogen addition treatments.

		DEEP			SHALLOW		
measure	**species**	**control**	**Nox**	**Nred**	**control**	**Nox**	**Nred**
shoot	*Lotus*	1.95 ±0.52	2.15 ±0.49	1.82 ±0.12	1.53 ±0.65	1.52 ±0.51	1.49 ±0.29
	Dactylis	0.95 ±0.35	0.99 ±0.47	0.75 ±0.41	0.43 ±0.09	0.46 ±0.10	0.48 ±0.13
	Silene	0.69 ±0.34	0.65 ±0.33	0.57 ±0.17	0.41 ±0.04	0.46 ±0.08	0.41 ±0.04
root	*Lotus*	1.25 ±0.44	1.42 ±0.32	1.04 ±0.20	1.26 ±0.52	1.00 ±0.12	1.02 ±0.47
	Dactylis	0.31 ±0.03	0.25 ±0.03	0.29 ±0.02	0.30 ±0.06	0.27 ±0.10	0.28 ±0.04
	Silene	0.21 ±0.04	0.21 ±0.03	0.21 ±0.02	0.22 ±0.05	0.19 ±0.03	0.19 ±0.02

Mixed effect models indicated that soil depth was a significant driver of shoot nitrogen content in *Dactylis* and *Silene*, in the nitrogen control group. When averaged over all levels of nitrogen addition treatments, soil depth had a significant influence on shoot tissue nitrogen content for all three species (see pairwise comparisons in Table 3.26). Root tissue nitrogen content was not found to be significantly influenced by soil depth or nitrogen treatment, nor by their interaction.

Table 3.26 Output of mixed effect models for species' tissue nitrogen content, and any significant pairwise comparisons.

model						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
LOTUS						
***Lotus* shoot tissue %N ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.4122	0.2422	20	-1.702	0.104	
Nox	0.2066	0.2422	20	0.853	0.404	
Nred	-0.1305	0.2422	20	-0.539	0.596	
shallow : Nox	-0.2186	0.3425	20	-0.638	0.530	
shallow : Nred	0.0863	0.3425	20	0.252	0.804	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	0.456	0.14	20	3.263	0.0039	

Table 3.26 continued over ...

... Table 3.26 continued

***Lotus* root tissue %N ~ F1 + F2 + F1:F2 + R1**						
shallow	0.0160	0.2374	24	0.067	0.947	
Nox	0.1700	0.2374	24	0.716	0.481	
Nred	-0.2103	0.2374	24	-0.886	0.385	
shallow : Nox	-0.4369	0.3358	24	-1.301	0.206	
shallow : Nred	-0.0296	0.3358	24	-0.088	0.930	
DACTYLIS						
***Dactylis* shoot tissue %N ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.5247	0.1797	20	-2.920	0.0085	**
Nox	0.0407	0.1797	20	0.226	0.8234	
Nred	-0.2009	0.1797	20	-1.118	0.2770	
shallow : Nox	-0.0106	0.2542	20	-0.042	0.9670	
shallow : Nred	0.2491	0.2542	20	0.980	0.3388	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	0.445	0.104	20	4.291	0.0004	
***Dactylis* root tissue %N ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.0042	0.0343	24	-0.123	0.903	
Nox	-0.0538	0.0343	24	-1.568	0.130	
Nred	-0.0180	0.0343	24	-0.526	0.604	
shallow : Nox	0.0260	0.0485	24	0.536	0.597	
shallow : Nred	-0.0047	0.0485	24	-0.097	0.923	
SILENE						
***Silene* shoot tissue %N ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.2750	0.1260	20	-2.183	0.0411	*
Nox	-0.0396	0.1260	20	-0.314	0.7565	
Nred	-0.1222	0.1260	20	-0.970	0.3436	
shallow : Nox	0.0849	0.1781	20	0.476	0.6390	
shallow : Nred	0.1212	0.1781	20	0.680	0.5042	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	0.206	0.0727	20	2.836	0.0102	

Table 3.26 continued over ...

... Table 3.26 continued

***Silene* root tissue %N ~ F1 + F2 + F1:F2 + R1**						
shallow	0.0080	0.0164	20	0.485	0.633	
Nox	0.0024	0.0164	20	0.148	0.883	
Nred	-0.0029	0.0164	20	-0.175	0.863	
shallow : Nox	-0.0285	0.0232	20	-1.230	0.233	
shallow : Nred	-0.0241	0.0232	20	-1.039	0.311	

Tissue carbon content varied widely depending on soil depth and nitrogen addition (Figure 3.22, Table 3.27). All species had lower shoot tissue carbon under the Nox treatment in deep soil, compared with the deep soil control, though lower than control in shallow soil. *Lotus* shoot tissue carbon was higher than the controls with Nred addition, in both deep and shallow soil treatments; tissue nitrogen in *Lotus* root tissue was higher than control in both nitrogen additions in the shallow soil treatment. *Dactylis* shoot carbon content was reduced with nitrogen-addition in deep soil, and higher than the control levels in shallow soil; root tissue nitrogen was higher in both nitrogen-additions in the deep soil, and with the Nox addition in shallow soil. *Silene* tissue nitrogen content was, for the most part, reduced in both shoot and root tissue with nitrogen-addition treatments, other than root tissue under Nred in shallow soil.

Lotus and *Dactylis* showed notable (near-significant) responses to soil depth and nitrogen addition treatments, when investigated via mixed effect models. *Dactylis* shoot tissue carbon varied its response to nitrogen treatment depending on soil depth (shallow: Nox, t = 2.039, 24 df, p = 0.0526). Change in *Dactylis* shoot percent carbon content was greater under oxidised nitrogen addition in shallow soils, compared to change in deep soil, or between control and Nred treatment in either soil depth. *Silene* shoot tissue carbon was significantly lower in the reduced nitrogen treatment compared with the control level (Nred, t = -1.907, 24 df, p = 0.0685) (Table 3.28).

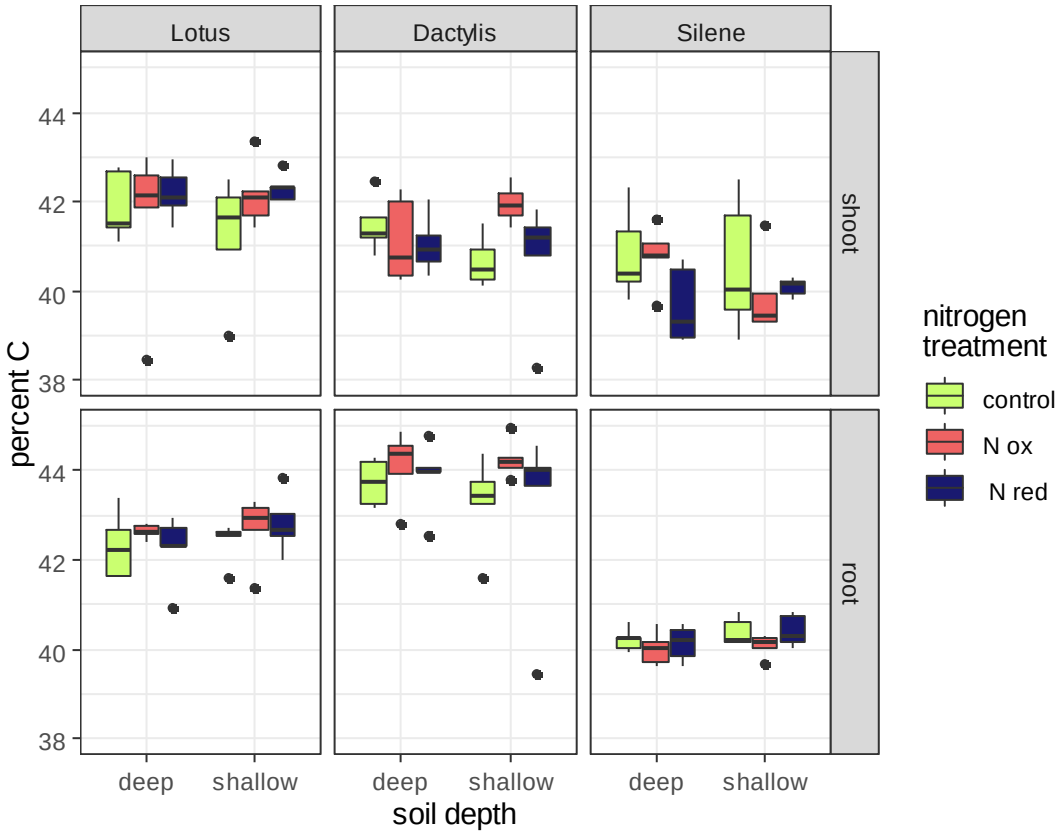


Figure 3.22 Plant tissue percent carbon content in post-experiment plants.

Table 3.27 Mean percent tissue carbon content across all soil depth and nitrogen addition treatments.

		DEEP			SHALLOW		
measure	**species**	**control**	**Nox**	**Nred**	**control**	**Nox**	**Nred**
shoot	*Lotus*	41.90 ±0.78	41.61 ±1.81	42.17 ±0.59	41.25 ±1.38	42.16 ±0.75	42.31 ±0.32
	Dactylis	41.48 ±0.63	41.13 ±0.95	41.04 ±0.65	40.67 ±0.56	43.91 ±4.10	40.70 ±1.41
	Silene	40.81 ±1.01	40.78 ±0.71	39.67 ±0.86	40.54 ±1.51	39.91 ±0.91	40.08 ±0.21
root	*Lotus*	42.31 ±0.74	42.63 ±0.17	42.25 ±0.78	42.40 ±0.46	42.69 ±0.77	42.83 ±0.67
	Dactylis	43.74 ±0.52	44.32 ±0.89	43.86 ±0.81	43.29 ±1.03	44.25 ±0.43	43.15 ±2.09
	Silene	40.21 ±0.25	40.02 ±0.38	40.14 ±0.40	40.39 ±0.31	40.07 ±0.24	40.41 ±0.32

Table 3.28 Output from mixed effect models for species' tissue carbon content. There were no significant pairwise comparisons.

model						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
LOTUS						
***Lotus* shoot tissue %C ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.6530	0.6725	24	-0.971	0.341	
Nox	-0.2989	0.6725	24	-0.431	0.670	
Nred	0.2741	0.6725	24	0.408	0.687	
shallow : Nox	1.2022	0.9510	24	1.264	0.218	
shallow : Nred	0.7849	0.9510	24	0.825	0.417	
***Lotus* root tissue %C ~ F1 + F2 + F1:F2 + R1**						
shallow	0.0931	0.3744	20	0.249	0.806	
Nox	0.3214	0.3744	20	0.859	0.401	
Nred	-0.0643	0.3744	20	-0.172	0.865	
shallow : Nox	-0.0382	0.5294	20	-0.072	0.943	
shallow : Nred	0.4876	0.5294	20	0.921	0.368	
DACTYLIS						
***Dactylis* shoot tissue %C ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.8124	1.2475	24	-0.651	0.5211	
Nox	-0.3510	1.2475	24	-0.281	0.7808	
Nred	-0.4437	1.2475	24	-0.356	0.7252	
shallow : Nox	3.5980	1.7642	24	2.039	0.0526	^
shallow : Nred	0.4780	1.7642	24	0.271	0.7887	
***Dactylis* root tissue %C ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.4487	0.7005	20	-0.641	0.528	
Nox	0.5809	0.7005	20	0.829	0.415	
Nred	0.1240	0.7005	20	0.177	0.861	
shallow : Nox	0.3850	0.9906	20	0.389	0.701	
shallow : Nred	-0.2637	0.9906	20	-0.266	0.792	

Table 3.28 continued over ...

... Table 3.28 continued

SILENE						
***Silene* shoot tissue %C ~ F1 + F2 + F1:F2 + R1**						
shallow	-0.2761	0.6003	24	-0.460	0.6498	
Nox	-0.0319	0.6003	24	-0.053	0.9580	
Nred	-1.1449	0.6003	24	-1.907	0.0685	^
shallow : Nox	-0.5971	0.8490	24	-0.703	0.4886	
shallow : Nred	0.6897	0.8490	24	0.812	0.4246	
***Silene* root tissue %C ~ F1 + F2 + F1:F2 + R1**						
shallow	0.1821	0.1809	20	1.007	0.326	
Nox	-0.1926	0.1809	20	-1.065	0.300	
Nred	-0.0685	0.1809	20	-0.379	0.709	
shallow : Nox	-0.1275	0.2558	20	-0.499	0.623	
shallow : Nred	0.0804	0.2558	20	0.314	0.757	

Plant biomass measures were assessed for correlations with tissue nitrogen and carbon contents, in order to gain insight into plant species' resource allocation strategies. *Dactylis* shoot biomass had a significant positive relationship with % nitrogen (Pearson's *rho* = 0.6582; t = 4.6265, 28 df, p = $7.695e^{-05}$). No biomass measure from *Lotus* nor *Silene* were found to have any significant correlations with tissue % nitrogen content.

3.4.7.2 Carbon:nitrogen (C:N) ratio

Mean root tissue C:N ratios were consistently higher than those of shoot material in all three species, across all treatment combinations; plants of all species were found to have higher shoot tissue C:N ratios in the shallow soil (Figure 3.23, Table 3.29).

All but two cases (*Dactylis* root tissue in oxidised nitrogen treatment, and *Silene* root tissue in the control group) had a higher C:N ratio in the shallow soil than in the deep soil mesocosms.

Table 3.29 Mean C:N ratios for shoot and root tissue for the three mesocosm species. For each case, n = 5.

		DEEP			SHALLOW		
species	**measure**	**control**	**Nox**	**Nred**	**control**	**Nox**	**Nred**
Lotus	shoot	23.16	20.01	23.33	31.47	31.06	29.54
	root	38.65	31.43	41.95	39.47	43.45	55.01
Dactylis	shoot	47.76	51.98	67.90	97.45	99.85	90.54
	root	144.19	177.38	152.78	148.74	175.95	157.26
Silene	shoot	70.71	75.47	75.18	98.91	89.13	98.19
	root	198.17	192.61	196.72	195.70	214.14	214.75

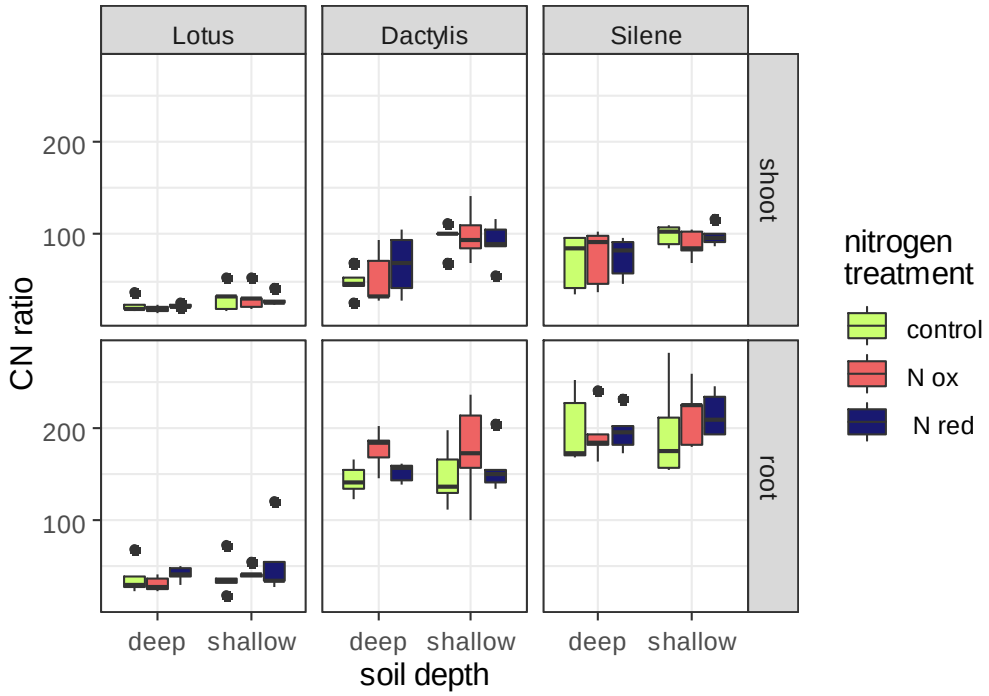


Figure 3.23 Influence of soil depth and nitrogen addition treatments on C:N ratio of shoot and root tissue for all species.

Tissue C:N ratios were investigated via mixed effect models (Table 3.30). Shoot tissue C:N ratios were higher in shallow soil compared with deep soil treatments; this was significantly different in *Dactylis* (t = 3.389, 20 df, p = 0.0029) and *Silene* (t = 2.193, 20 df, p = 0.0403), and near-significant in *Lotus* (t = 1.811, 20 df, p = 0.0851). *Dactylis* root CN ratio was higher under the Nox treatment in both soil depths, compared with both the control and the Nred treatment; the difference in comparison to the control CN ratio was significant in deep soil (t = 3.158, 12 df, p = 0.0083) but not in shallow soil.

Table 3.30 Output from mixed effect models of CN ratio responses to soil depth and nitrogen addition treatments.

model						
term	**estimate**	**SE**	**df**	**t-value**	**p**	
LOTUS						
***Lotus* shoot CN ratio ~ F1 + F2 + F1:F2 + R1**						
shallow	8.3158	4.5911	20.00	1.811	0.0851	^
Nox	-3.1441	4.5911	20.00	-0.685	0.5013	
Nred	0.1762	4.5911	20.00	0.038	0.9698	
shallow : Nox	2.7283	6.4928	20.00	0.420	0.6788	
shallow : Nred	-2.1043	6.4928	20.00	-0.324	0.7492	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	-8.52	2.65	20	-3.216	0.0043	
***Lotus* root CN ratio ~ F1 + F2 + F1:F2 + R1**						
shallow	0.8224	12.5182	19.65	0.066	94.83	
Nox	-7.2241	12.5182	19.65	-0.577	0.5704	
Nred	3.3027	12.5182	19.65	0.264	0.7947	
shallow : Nox	11.1975	17.7034	19.65	0.633	0.5344	
shallow : Nred	12.2328	17.7034	19.65	0.691	0.4977	
DACTYLIS						
***Dactylis* shoot CN ratio ~ F1 + F2 + F1:F2 + R1**						
shallow	49.694	14.662	20.00	3.389	0.0029	**
Nox	4.227	14.662	20.00	0.288	0.7761	
Nred	20.146	14.662	20.00	1.374	0.1846	
shallow : Nox	-1.824	20.735	20.00	-0.088	0.9308	
shallow : Nred	-27.058	20.735	20.00	-1.305	0.2067	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	-40.1	8.46	20	-4.733	0.0001	

Table 3.30 continued over ...

... Table 3.30 continued

***Dactylis* root CN ratio ~ F1 + F2 + F1:F2 + R1**						
shallow	4.547	19.14	24.00	0.238	0.8142	
Nox	33.193	19.14	24.00	1.734	0.0957	^
Nred	8.596	19.14	24.00	0.449	0.6573	
shallow : Nox	-5.976	27.07	24.00	-0.221	0.8271	
shallow : Nred	-0.069	27.07	24.00	-0.003	99.80	
SILENE						
***Silene* shoot CN ratio ~ F1 + F2 + F1:F2 + R1**						
shallow	28.195	12.857	20.00	2.193	0.0403	*
Nox	4.755	12.857	20.00	0.370	0.7154	
Nred	4.474	12.857	20.00	0.348	0.7315	
shallow : Nox	-14.533	18.183	20.00	-0.799	0.4445	
shallow : Nred	-5.189	18.183	20.00	-0.285	0.7783	
Model pairwise comparisons						
comparison	estimate	SE	df	t-ratio	p	
deep – shallow	-21.6	7.42	20	-2.913	0.0086	
***Silene* root tissue CN ratio ~ F1 + F2 + F1:F2 + R1**						
shallow	-2.467	16.021	20.00	-0.154	0.879	
Nox	-5.560	16.021	20.00	-0.347	0.732	
Nred	-1.441	16.021	20.00	-0.090	0.929	
shallow : Nox	23.999	22.657	20.00	1.059	0.302	
shallow : Nred	20.491	22.657	20.00	0.904	0.377	

3.4.8 Productivity measures as a reflection of species' moisture and nutrient preferences

The relative importance of species' preferences for moisture and nutrient levels, as indexed by Ellenberg F and N values (Hill et al, 1999), were considered as possible factors influencing species' responses to soil depth and nitrogen form and availability. Between the three species in the mesocosm communities, there were two levels of moisture (F) values, and three nutrient (N) levels (Table 3.31). Ellenberg nutrient N levels corresponded uniquely one to each species, so any analysis of this was representative of differences in the species' responses, and would be covered by other analysis, e.g. into shoot biomass response.

Table 3.31 Ellenberg indicator values for species preferences for moisture (F) and nitrogen (N) (Hill *et al.*, 1999).

Ellenberg moisture, F		**Ellenberg nutrient, N**	
level	**species**	**level**	**species**
F4	*Lotus corniculatus*	N3	*Lotus corniculatus*
	Silene vulgaris	N5	*Silene vulgaris*
F5	*Dactylis glomerata*	N6	*Dactylis glomerata*

Analysis of Ellenberg moisture levels on shoot and root biomass showed that patterns of response to the Ellenberg levels reflected *Lotus* patterns of response, apart from the influence of soil depth on root biomass in the water-only control group. In this case, *Lotus* root biomass showed little difference between soil treatments (mean root biomass in deep soil was 24.0 g, in shallow soil 25.3), whereas *Silene* produced significantly more root in the deep soil group (mean 22.2 g) compared to that in the shallow soil group (mean 13.3 g). With only three species under consideration, and five Ellenberg index levels between the two factors, it is doubtful that using Ellenberg values is an appropriate basis for further analysis of this restricted community.

3.4.9 Soil carbon and nitrogen content

Mean pre-experiment levels of soil carbon and soil nitrogen:

- carbon, C, 0.63 mg
- nitrogen, N, 0.014 mg

There was a strong correlation between post-experiment soil carbon and nitrogen content in individual mesocosms (Pearson's $r = 0.95$, $p < 0.001$). Soil C:N ratios were consistently lower than those for both plant tissue types.

Post-experiment soil nutrient content was investigated for all mesocosms; carbon content was significantly greater in shallow bins under the control nitrogen treatment (shallow mean 1.02 mg, deep 0.58 mg; $p < 0.05$), but soil depth had no significant influence on carbon content under either of the nitrogen-addition treatments (Figure 3.24). Nitrogen content was not significantly influenced by any combination of soil depth or nitrogen treatment. There were, however, measurable differences in nutrient levels, which were investigated in relation to plant biomass measures, to assess possible associations between nutrient acquisition and plant resource partitioning.

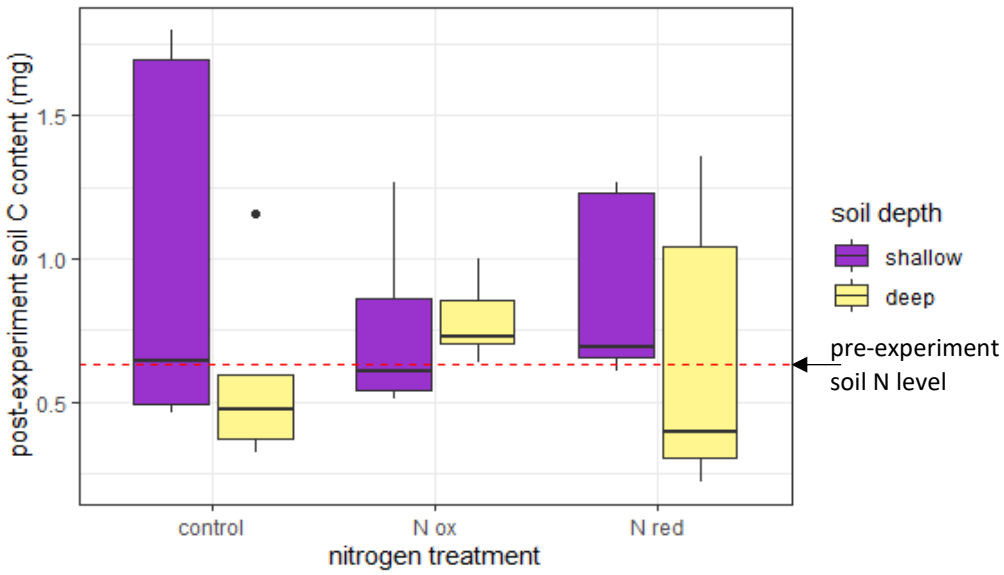


Figure 3.24 Post-experiment soil carbon content. Pre-experiment mean carbon content (0.63 mg) is indicated by dashed red line.

The majority of mesocosms (n = 21) had an increased soil nitrogen content, and 17 mesocosms had increased soil carbon, at the end of the experiment. Figure 3.25 and Figure 3.26 illustrate the final soil nitrogen associated with the different soil depth and nitrogen-addition treatments, along with the pre-experiment reference baseline.

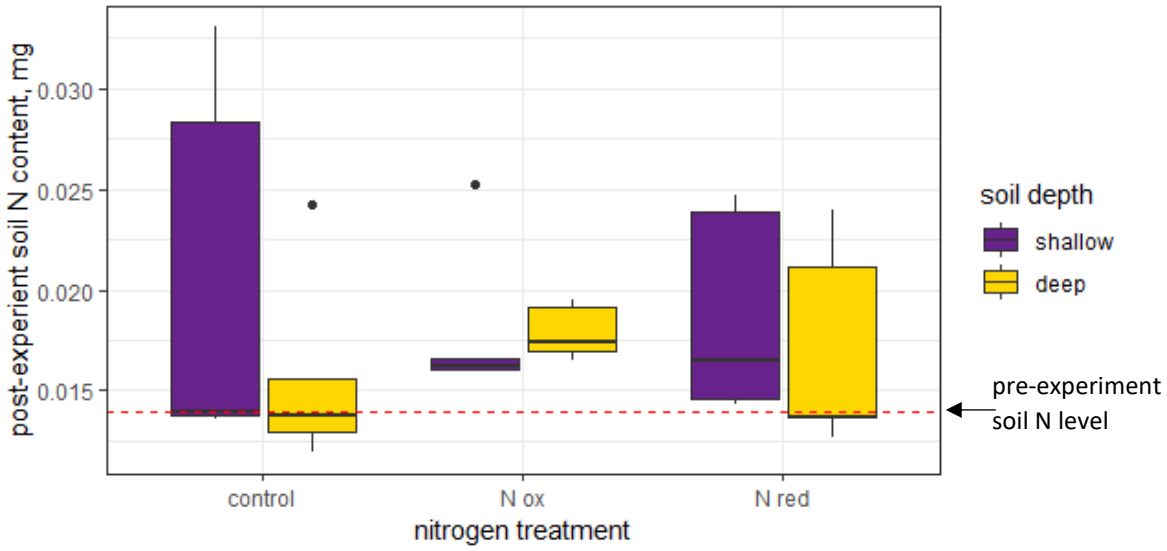


Figure 3.25 Post-experiment soil nitrogen content in the two soil depth treatments (n = 30). Pre-experiment soil nitrogen level (0.014 mg) is indicated by the dashed red line.

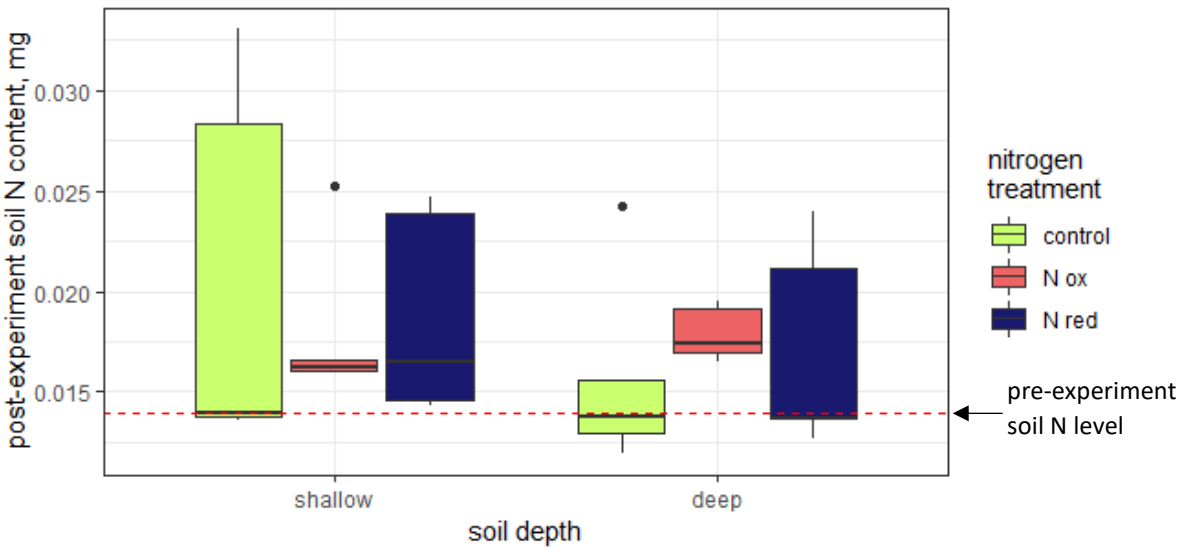


Figure 3.26 Post-experiment soil nitrogen content in the three nitrogen-addition treatments. Pre-experiment soil nitrogen level (0.014 mg) is indicated by red dashed line.

Changes in soil nitrogen and carbon levels were calculated by subtracting the pre-experiment values from the post-experiment values, giving deltaN and deltaC values (Figure 3.27) (where delta is used to indicate "change in").

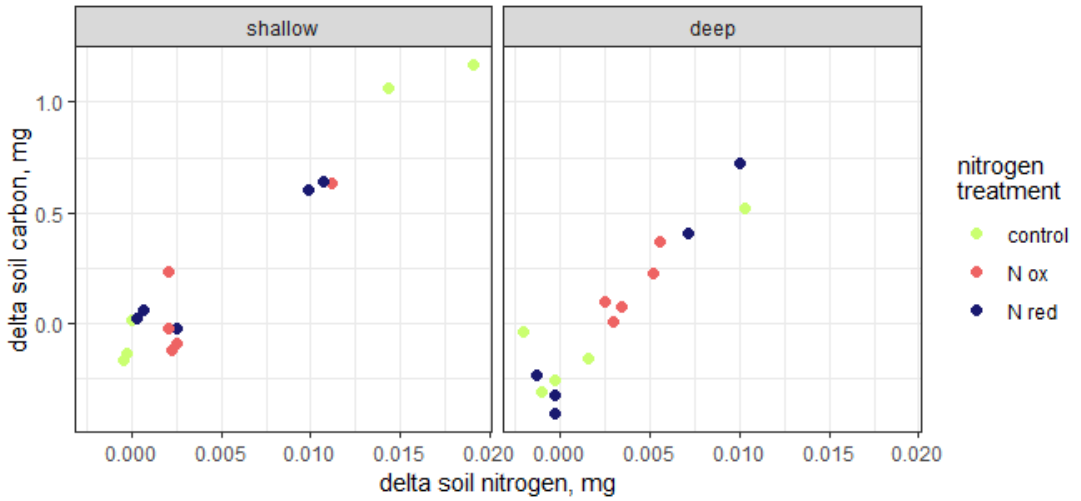


Figure 3.27 Changes in mesocosm soil nitrogen, N, and carbon, C, given as delta Δ values.

Mesocosms with a net reduction in both nitrogen and carbon (n = 5) were in the control and reduced nitrogen treatments. All mesocosms in the oxidised nitrogen group had an increase in soil nitrogen at the end of the experiment compared to the baseline (pre-experiment) value; of these, three mesocosms had a net reduction in soil carbon, and all of these were in the shallow soil group. Mesocosms with enhanced post-experiment soil nutrient levels were spread across all treatment combinations.

3.4.9.1 *Soil nutrient depletion associated with plant productivity*

Post-experiment soil nitrogen content in each mesocosm showed an overall negative trend when plotted against aggregated total biomass data (i.e. soil nutrient levels declined as total mesocosm biomass increased). None of the species' shoot biomass was significantly correlated with post-experiment soil nitrogen at $p < 0.05$ level, though *Lotus* shoot biomass showed a moderate ($p = 0.06$) association with post-experiment soil nitrogen in the deep soil treatment. *Lotus* root biomass had a significant negative relationship with soil nitrogen content ($p < 0.05$) (Figure 3.28) in the shallow soil treatment, and a non-significant positive ($p = 0.1394$) one in the deep soil treatment. Both *Dactylis* biomass measures had contrasting relationships with soil depth, being positive in deep soil and negative in shallow soil.

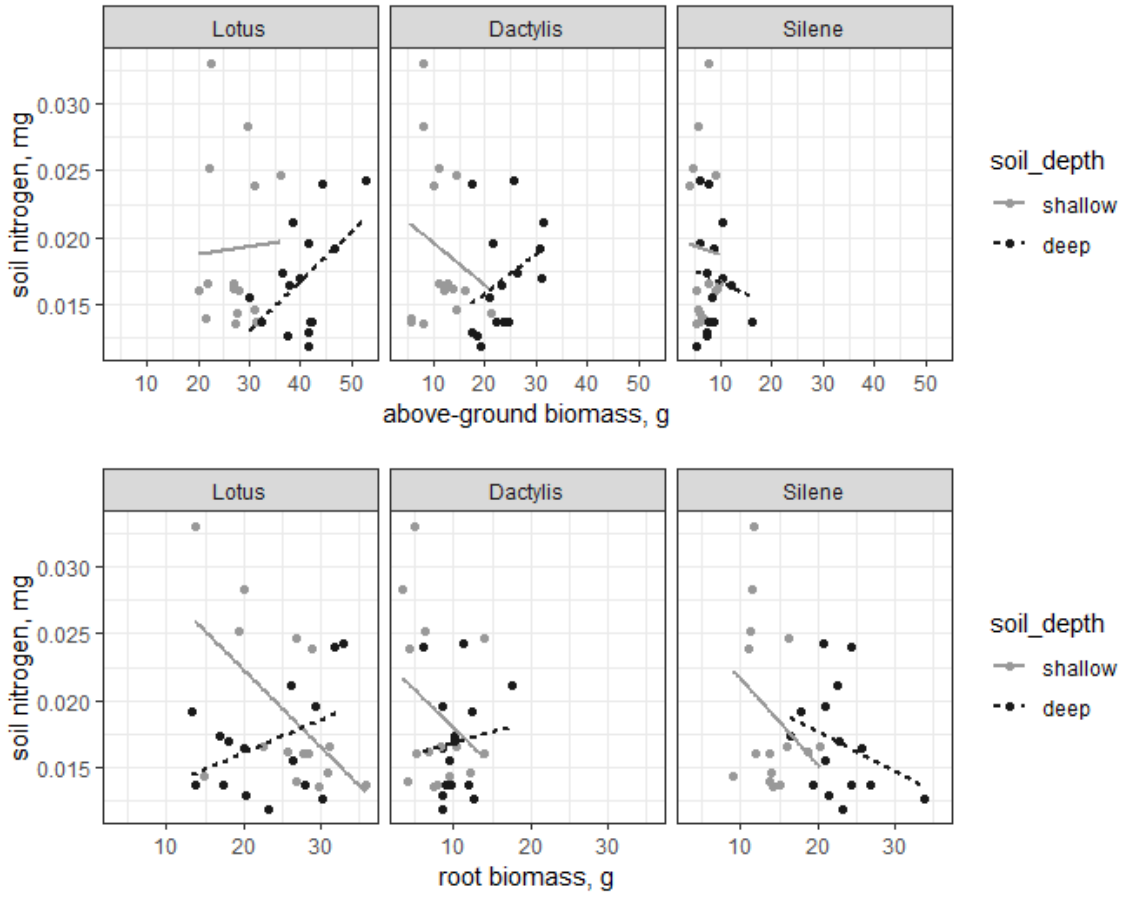


Figure 3.28 Post-experiment soil nitrogen content relationship with above-ground (shoot) and root biomass.

3.5 Discussion

3.5.1 Biomass response to soil depth (hypothesis H3.1)

As expected, greater soil depth promoted increased biomass across the board; total biomass per mesocosm was significantly greater in deep soil, as were total mesocosm shoot and root biomass. This agrees with findings from previous studies that considered the role of soil depth on grassland species (Berendse, 1981; Dornbush and Wilsey, 2010; Fridley *et al.*, 2011; Selmants, Zavaleta and Wolf, 2014), and which suggest that soil depth is positively correlated with both productivity and resource availability.

The underlying assumption in hypothesising that soil depth would promote increased biomass was that increased soil depth would provide a larger spatial niche, as per Dimitrakopoulos and Schmid (2004), with larger pools of soil moisture and nutrient held therein. Levels of plant-available nutrients are predominantly controlled by soil microbes engaged in nitrogen mineralisation, and it is well understood that the microbes responsible for nitrogen mineralisation are sensitive to temperature and require adequate soil moisture to function (e.g. Boring *et al.*, 1988; Jackson, Schimel and Firestone, 1989; Bever *et al.*, 2010); thus, soil moisture impacts on plant-available levels of nitrogen and, as a consequence, nitrogen fluxes within an ecosystem. Microbial activity is also affected by soil pH, and mineralisation and nitrification rates have been shown to have optimum ranges at high pH levels (mineralisation between 6.0 to 8.0, nitrification between 7.5 to 8.0) (Aciego Pietri and Brookes, 2008). The growing medium had a starting pH of c. 8.4, comparable to that under natural temperate calcareous grasslands, so good rates of mineralisation were expected, provided that soil moisture levels remained adequate, thereby optimising plant-available nitrogen.

At harvest and excavation in July 2018, root mass within the shallow soil mesocosms was seen to be crowded; the basal root mass that was seen to have formed in all the mesocosms took up proportionately more of the spatial niche, where roots were closely bundled together. In order to explore differences in mesocosm biomass, it is useful to briefly consider the effect of close crowding of roots in this way. Root restriction naturally has a dwarfing effect through the interaction of a number of morphological and physiological factors. The effect of root restriction has been particularly well-studied in trees (e.g. Beidler *et al.*, 2014; Ow and Ghosh, 2017) in both wild and cultivated contexts, and in horticulturally important species such as tomatoes (Peterson, Reinsel and Krizek, 1991; Hameed, Reidfj and Rowe, 2017), peppers (Ismail and Davies, 1998) and cotton (Carmi, 1986), but less so in natural or semi-natural habitats or wild species.

Issues with comparing the responses of horticultural species with native grassland species are predominantly those of trait expression, whereby organisms interact with each other and with their environment (Van de Waal *et al.*, 2018). Chapin (1980) considered herbaceous crop plants to be comparable to wild plants characteristic of more fertile environments, in terms of their trait responses to water and nutritional fluctuations. Both groups have relatively high growth and nutrient-supply response rates; nutrient stress responses include increased root:shoot ratio with increased root absorption capacity, and decreased photosynthesis and allocation to reproduction. In comparison, native grassland species inhabit relatively less fertile environments. They typically have lower root absorption capacity and maximise nutrient uptake through a higher root:shoot ratio, though with reduced plasticity in their ability to change their root:shoot allocation pattern, in line with Grime's stress-tolerator strategy (Grime, 1977).

Common to all root-restriction experiments is a general decrease in biomass but no significant reduction in flowering (Carmi, 1986; Peterson, Reinsel and Krizek, 1991); root-restricted cotton plants have been seen to flower sooner (Carmi, 1986), which is interestingly also the nutrient - deficit response of cotton in field situations. Phenological stages were not noted during the mesocosm experiment, so no comment can be made regarding flowering onset in the different soil depth treatments. Investment in flowering organs was measured in June 2018, and a significantly higher number of flower units counted from *Dactylis* and *Silene* growing in deep soil. This contradicts the above generalisation regarding the effect of root restriction on flowering effort, and is interpreted as being due to the differential responses seen in species characteristic of different fertility levels, i.e. from the fact that the above studies involve horticulturally important species, whereas those involved in the mesocosm experiment are native grassland species. *Dactylis* particularly could be considered a long-lived competitive species, and as such, would be expected to respond to nutrient stress by reduced allocation to reproduction (Chapin, 1980b).

Although there is a consensus that root restriction leads to reduced biomass, there is debate over the mechanisms involved. In experiments where roots were restricted and water and nutrient levels maintained (Ismail and Davies, 1998; Graham and Wheeler, 2015), biomass was still seen to be lower than in unrestricted control plants, which refutes arguments that root restriction interferes with plant-water balance (e.g. as proposed by Hameed *et al.* (1987)). Ismail and Davies (1998) compared root-restricted plants with unrestricted controls and a further unrestricted group undergoing gradual water deficit, and found leaf water potential in

restricted plants comparable to that in the control plants, and greater than in those in the drying group, which indicated that the observed reduction in plant growth seen in the root-restricted plants was not due to water deficit. Most of these studies have considered physiological processes that are beyond the scope of this experiment, and a range of chemical and hormonal drivers have been proposed as being actively involved in the observed responses.

The results from the mesocosm experiment adds to this diverse body of data from both lab and field studies, and indicate that soil depth is positively correlated with biomass production, though the mechanisms responsible are still to be untangled.

3.5.2 Productivity response to nitrogen treatment (hypotheses H3.2, H3.3)

The influence of nitrogen availability on plant productivity is well-established and documented, as is its dependence on soil moisture (e.g. Silvertown *et al.*, 1994; Phoenix *et al.*, 2008; Du *et al.*, 2014; Pallett, Pescott and Schäfer, 2016; Saud *et al.*, 2017). In general, increased soil nitrogen availability is correlated with increased productivity, though species' responses vary depending on their phenology, life stage, resource acquisition and use efficiencies, and adaptive ability (Grime, 1977; Chapin, 1980b), and it is widely thought that differential responses to nitrogen availability at an individual species level are believed to effect changes in plant community composition (Inouye and Tilman, 1995; Wardle, Bonner and Barker, 2000; Lamb, Kembel and Cahill, 2009; Emmett *et al.*, 2011; Field *et al.*, 2014). As the pre-experiment total soil nitrogen of the growing medium was 0.06%, it was felt that this would provide a low-nutrient starting point against which to assess changes in biomass resulting from the nitrogen addition treatments. Equally, all plants were well beyond seedling age at commencement of the experiment, so the higher nitrogen requirements of that early life stage would not have been a confounding factor in subsequent response to nitrogen availability (Tulloss and Cadenasso, 2016).

Nitrogen addition rates in the treatments were low, at an equivalent of 25 kg N ha^{-1} yr^{-1}, which, in addition to the background atmospheric nitrogen deposition experienced at Walton Hall, brought the total annual equivalent to 43.2 N ha^{-1} yr^{-1}, for those months when the treatment was being applied. This pushed the total nitrogen deposition on the mesocosms beyond the currently accepted critical load for calcareous grassland - the critical load is the level below which deposition of a substance has no long-term harmful effect on ecosystem function or structure, so far as current knowledge allows. Critical load for calcareous grassland was set in 2002 as 15-25 kg N ha^{-1} yr^{-1} (Bobbink *et al.*, 2003), and later revised down to 20 kg N ha^{-1} yr^{-1} for UK calcareous grasslands, referencing field and experiment observations of ecosystem changes

(Emmett *et al.*, 2011; Hall *et al.*, 2011). Prolonged and chronic nitrogen deposition has been found to have a measurable effect on many ecosystems globally (Chapin, 1980a; Stevens *et al.*, 2006, 2009; Maskell *et al.*, 2010; Field *et al.*, 2014; Ma *et al.*, 2016; Wilkins, Aherne and Bleasdale, 2016), resulting in eutrophication, acidification and reduced diversity. The cumulative effects of deposition are thought to be particularly damaging to calcareous grasslands (Stevens *et al.*, 2006; Phoenix *et al.*, 2008; Emmett *et al.*, 2011; JNCC, 2011; Diekmann *et al.*, 2014).

Although the experiment only ran over two partial growing seasons, there was sufficient difference in nitrogen deposition between the nitrogen addition treatments, to see a measurable response from the mesocosm species. Total mesocosm biomass, as harvested at the end of the second growing season, was greater in both nitrogen-addition treatments compared to the control treatment (reduced nitrogen mean 57.79 $\pm$2.85 g; oxidised nitrogen mean 55.39 $\pm$3.12 g; control mean 52.72 $\pm$3.35 g). This indicates that, at a mesocosm community level, there was a positive trend between increased nitrogen and biomass, which is expected for nitrogen-limited environments (Grime, 1977; Cahill, 2002). This is interesting with regard to nitrogen critical load exceedance, as damage to habitats lies in the change in species composition or functional composition, rather than being indicated by an increase in biomass. Increased growth by competitive grasses, for example, can competitively exclude low-growing calcareous grassland specialist species, thus reducing species richness despite potentially increasing productivity.

Biomass responses at a species level were varied, illustrating the differences in species' preferences. In order to address the question of whether increased nitrogen *per se* influenced biomass, data for the nitrogen-addition and soil depth treatments were considered together. When data were pooled for the two nitrogen-addition treatments, nitrogen addition generally (but not significantly) increased total biomass in *Dactylis* and *Silene*, but not for *Lotus*. At a species level, nitrogen addition had no significant effect on any biomass measure when both soil depth treatments were pooled, but was linked to varied responses by species when soil depth was taken into account. *Dactylis* had the strongest response, with a near-significant response in shoot biomass (Nox: t = 2.030, 27 df, p = 0.0523; Nred: t = 1.766, 27 df, p = 0.0887), and a significant response by root biomass in the Nred treatment (t = 2.162, 23 df, p = 0.0413) compared to the control. In contrast, *Lotus* was less productive with additional nitrogen in deep soil compared to the control group, and only increased total biomass with nitrogen addition in shallow soil.

Plant biomass responses are determined by the most limiting resource, with the degree of nutrient limitation being broadly indicated by the strength of the response when the limiting nutrient is applied (Chapin, 1980b). Both *Dactylis* and *Silene* are perennial species that rate moderately high on the Ellenberg nitrogen index (*Dactylis* N6, *Silene* N5), indicating a preference for a higher nutrient availability, and the observed increase in productivity in the nitrogen-addition treatments would confirm that *Dactylis* and *Silene* were nitrogen-limited. *Lotus* is a legume, forming N_2-fixing symbiosis with bacterial rhizobia, and would therefore not be expected to be nitrogen-sensitive; it has been shown to decline along the UK gradient of nitrogen deposition in acid grasslands (Emmett *et al.*, 2011) due to being out-competed by grass species when nitrogen is abundant; *Lotus* was also seen to track levels of atmospheric nitrogen deposition in the Park Grass long-term field experiment at Rothamsted (Storkey *et al.*, 2015). Its status in calcareous grasslands is currently unknown. The general decline in total biomass seen in *Lotus* with nitrogen addition is in keeping with its preference for low nutrient environments, as indicated by its low Ellenberg indicator value for nutrient (Ellenberg N of 3 (low)).

3.5.3 Biomass allocation (root:shoot) response to soil depth and nitrogen addition (hypothesis H3.4)

There are changes in biomass allocation that take place as part of normal growth and development; proportionately more resources are allocated to root development in the seedling and early growth stage, giving a high root:shoot ratio, which declines as plants mature (McConnaughay and Coleman, 1999). By leaving the mesocosm experiment to run over two seasons gave ample time for such differences associated with very early life stages to be erased, and for variation in root:shoot ratios to reflect responses to differences in the environmental variables of soil depth and nitrogen availability. When resource-limited, plants develop slowly and retain the higher root:shoot ratios of immature plants (McConnaughay and Coleman, 1999); this is particularly true of perennial species, which respond to nitrogen stress by reduced shoot production and a reduced allocation of resources to reproduction (Chapin, 1980b). Conversely, fast-growing annual species may be prompted to bring forward flowering by stress, as a stress-avoidance strategy operating at species level. In all three mesocosm species, root:shoot ratios were negatively correlated with the number of flowering plants. In total, 86 plants out of 270 (i.e. 32%) were not flowering when surveyed in mid June; of these, a greater proportion of non-flowering plants of each species were in shallow soil.

There are also changes in biomass allocation that are associated with changing levels of factors such as soil moisture and nutrients; generally, increasing water and nutrients lead to greater biomass production. It has been widely observed that increasing nutrient availability leads to

increased shoot biomass, and that nutrient stress leads to a greater proportion of resources being allocated to roots to seek out more nitrogen; if water is limiting, a lower proportion of resources are allocated to shoots – in terms of biomass allocation, the result is effectively the same, though possibly driven by different mechanisms. Root:shoot ratios can indicate whether water and nutrient availabilities are adequate, and allocation to different structures - i.e. choosing to prefer leaves (photosynthesis) over roots (acquisition of other resources) - will directly affect a plant's competitive ability (Tilman, 1987).

Comparisons of mesocosm root:shoot ratios between nitrogen-addition and soil depth treatments were generally consistent with predictions based on optimal partitioning theory – biomass allocation to roots decreased under higher nutrient conditions (i.e. with nitrogen addition). Root:shoot ratio (R:S) also indicated that there was a significantly higher allocation overall to shoot biomass in deep soil (mean R:S 0.79) compared to shallow soil (mean R:S 1.06), suggesting that the mesocosm as a whole was less limited in deep soil, as was expected.

Individual species' responses suggest that not only are soil depth and nitrogen addition interacting, but that nitrogen form and individual species' strategies for resource acquisition and allocation were modifying the results further. Root:shoot ratios were all significantly influenced by soil depth, but nitrogen treatment was not a significant driver of differences at any level (mesocosm, species) or in interaction with soil depth. *Lotus* and *Dactylis* responded to shallower soil by increasing their root:shoot ratios, indicating an increase in root foraging output compared to investment in shoot biomass and photosynthesis. In contrast, *Silene* reduced its root:shoot ratio in shallow soil, though the magnitude of change was less that by which *Lotus* and *Dactylis* had increased.

In terms of the interaction of soil depth and nitrogen form on root:shoot ratios, species' responses were consistent in the deep soil treatment, with all three species reducing their root:shoot ratio under the oxidised nitrogen treatment, and increasing the root:shoot ratio with reduced nitrogen. This suggests that all three species were able to utilise the additional oxidised nitrogen in deep soil, but were resource-limited with the addition of reduced nitrogen. In shallow soil, this pattern of response changed, with all soil depth-nitrogen form treatment combinations seeing a reduction in the root:shoot ratio apart from *Lotus* under oxidised nitrogen, where the root:shoot ratio was increased. This suggests that *Lotus* was more nitrogen-limited in shallow soil, and switched preference to reduced nitrogen in these conditions. A reduction in above-ground biomass is considered the commonest response to

water deficit (Castillo *et al.*, 2017), and it is likely that the reduction in shoot biomass seen in *Lotus* in shallow soil reflects this.

It has been seen that biomass partitioning responses are influenced by complex interactions between soil depth, nitrogen availability and species' individual strategies. Though the mechanisms behind those partitioning responses remain complex; the hypothesis that root:shoot ratio reflects plant responses to water and nutrient status is supported. Of particular interest is the wide variation in species' responses to soil depth-nitrogen availability interactions, indicating that species' identity is an important factor.

3.5.4 Tissue element response to nitrogen addition (hypothesis H3.5)

Ecological stoichiometry theory predicts that enhanced soil nitrogen availability results in an increase in the relative abundance of nitrogen in plant tissues, and therefore shoot and root C:N ratios were expected to be reduced in the nitrogen-addition treatments. This was not wholly borne out by my results, which indicated that soil depth was interacting with nitrogen addition to modify species' responses, and that species' identity operating to further modify resource allocation responses.

Lower water availability normally results in reduced uptake of nutrients and reduced tissue concentrations. Increased plant C:N has been correlated with lower water availability (Zhou, Talley and Luo, 2009; Sardans, Rivas-Ubach and Peñuelas, 2012; Luo *et al.*, 2017), which agrees with the general trend observed in root and shoot C:N in the mesocosm plants. The mesocosm data show that most plant tissues have lower %N content in shallow soil, indicating that the plants are unable to access or use soil nitrogen, even in the nitrogen-addition treatments; the suggestion being that water limitation in shallow soil is inhibiting absorption of available soil nitrogen. Carbon content was also lower in control plants in shallow soil, which is in line with Luo *et al.* (2017) finding reduced shoot carbon after exposure to drought stress. Decreased root and shoot C:N has been associated with increased nitrogen availability (Heyburn *et al.*, 2017), and here, was mainly associated with deep soil.

The rapid growth in biomass attributed to increased nitrogen availability makes plants more vulnerable to drought damage (Chapin, 1980b, 1991), and increases in tissue nitrogen increases the probability of tissue damage from biotic and abiotic factors (Phoenix et al., 2012). *Lotus'* increased shoot nitrogen content and reduced root:shoot ratio in the oxidised nitrogen treatment in deep soil left it vulnerable to summer drought. As already discussed, the general

response to nitrogen addition, when the water supply is adequate, is to increase biomass, and particularly to increase shoot biomass with enhanced tissue nitrogen content. Unexpected extreme summer temperatures in June 2018 resulted in noticeable leaf scorching in *Lotus* plants in deep soil, as soil moisture supplies were inadequate to support the increased biomass. *Lotus* plants in adjacent shallow soil mesocosms, though experiencing the same temperatures and solar radiation, were affected much less. The two mesocosms in Figure 3.29, below, are deep-Nox and shallow-control treatment combinations. Deep-Nox had increased tissue nitrogen in both root and shoot, and a lower root:shoot ratio; shallow-control had decreased shoot nitrogen and an increased root:shoot ratio; similar patterns of scorching were found across all the mesocosms, with those in the deep soil treatment suffering an apparently greater degree of scorching. Despite greater soil water reserves in the deep soil, the proportionally greater investment in shoot growth rendered the plants vulnerable to drought, using the water reserves more quickly and being unable to sustain the greater shoot biomass.

Figure 3.29 Contrasting response to a short-term episode of extreme temperature in June 2018.

Biomass nitrogen content was seen to increase with soil depth, in this and other studies (e.g. Selmants, Zavaleta and Wolf, 2014), and is implicated in the degree of scorching seen as a result. The effect of this unexpected extreme summer drought illustrates one mechanism whereby perturbations in climate and weather patterns may act in concert with other factors to

drive changes in grassland communities, and how soil depth may impact plant communities in unexpected ways.

3.5.5 Species' response to nitrogen form

All three species used in the mesocosm experiment are considered to be deep-rooted, with similar potential rooting depths beyond the 20 cm maximum depth allowed by the mesocosms. There were observed but unmeasured differences in their root architecture – *Lotus* and *Dactylis* had long, coarsely fibrous roots with many rooting points, which formed a dense, fibrous mat of fine roots in the base of the soil layer. *Silene*, in contrast, appeared to be strongly geotropic; multiple tap roots from each crown were formed, which were thickened and robust, with a strong vertical habit. The thick taproots reached the base of the soil layer, but terminal fine roots were less extensive than those of *Lotus* and *Dactylis*, and they did not circumnavigate the extent of the base of the soil layer.

Species' responses to nitrogen form have already been partly dealt with in the sections on biomass generally and element allocation, and have been seen to vary widely depending on species identity, and the interaction of soil depth and nitrogen form. To summarise: *Dactylis* increased all biomass measures with both nitrogen forms, across both soil depths, which is in keeping with its generalist/competitor nature. *Lotus* biomass is inhibited by increased availability of oxidised nitrogen in deep soil, but able to benefit from additional reduced nitrogen more readily in shallow soils where a higher proportion of biomass is allocated to shoot development. *Silene* increased biomass production in shallow soils when both forms of nitrogen were added, also with a higher proportion of biomass being allocated to shoot production, whereas in deep soil, *Silene* favoured shoot growth with the addition of oxidised nitrogen, and root growth with the addition of reduced nitrogen.

Species' responses to nitrogen form are very different: species identity is important in the responses, and allocation of nutrients is very plastic, being influenced by the nitrogen form-soil depth interaction. In deep soil, all species followed the same pattern of change in proportions of shoot and root biomass, compared to that in the control group; in the oxidised nitrogen treatment, all species' shoot biomass was greater than control, and root biomass lower than control. This indicates an increased proportional allocation to shoot biomass, suggesting that the plants were nitrogen-limited, and responding to increased available nitrogen as nitrate

(NO_3^-). In the reduced nitrogen treatment, this pattern was reversed, in that all three species had a lower proportion of shoot biomass compared to control, and a higher proportion of root biomass.

3.5.6 Conclusions

Interpreting species' responses makes sense only in the context of knowledge of their characteristics, habits and requirements. Individual species' total biomass reflected differences not only in their preferences for chemical and physical environment, but also differences in their life form and strategies: *Lotus* consistently produced greater biomass than either *Dactylis* or *Silene*, which both have an upright form; *Lotus* has a low-growing, bushy growth form, which roots readily from stem contact with the ground, producing a dense mat of vegetation. *Dactylis* is highly competitive so has higher needs for nutrient and moisture, whereas *Lotus* and *Silene* prefer low nutrient, drier habitats.

Soil depth, soil moisture and nitrogen availability are intimately interconnected as factors influencing plant growth and development, and interact with species' individual strategies. There is compelling evidence that soil depth is a major factor in not only productivity of temperate grasslands, at which this study was aimed, but also plays an important role in ecosystem carbon and nitrogen cycles. The mechanisms are complex, and require the consideration of other factors such as biomass allocation (root:shoot ratio) and within-tissue concentrations of carbon and nitrogen to indicate which resource requirements are being fulfilled by deeper soil, for particular species or plant communities. Although the role of soil depth *per se* has not been studied extensively, it is an important component of the physical niche that fulfils a number of essential roles for plant communities, and, as such, has potential to be an important player in nitrogen and carbon cycling, habitat conservation and competitive interactions within associated plant communities.

Given the paucity of previous work, there is scope for further research into the modifying influence of soil depth on productivity and performance of calcareous grassland species. There is an undoubted strong signal for a response to soil depth in biomass and C:N ratio. The response to nitrogen form is complicated, as both soil depth and species' identity are important factors. The allocation of nutrients is a very plastic response and is shown here to be influenced by nitrogen form and soil depth. From this evidence, it would seem that species' responses to the combined effects of soil depth and nitrogen addition cannot be generalised.

Chapter 4 The influence of soil depth and other environmental variables on species richness and community composition of calcareous grassland plant communities

In this chapter, I present the results of field surveys carried out on eleven calcareous grasslands in the UK, and associate species and functional composition with prevailing or recent climatic conditions. I also attempt to assess the role of soil depth in community responses as a potential mediator of the impacts of climate change and atmospheric nitrogen deposition.

Species richness responses to environmental variables were often not straightforward, and presented results that were challenging to interpret. Soil depth metrics appear to be important factors for the maintenance of species richness on some sites, with the degree of variation in soil depth across a site (the soil depth range, i.e. the difference between maximum and minimum observed soil depths) being as important as larger scale metrics such as mean soil depth across a site in terms of relevance for diversity.

4.1 Introduction

Limestone grasslands occur predominantly on well-drained, shallow soils that dry out quickly in summer. High mean summer temperature and low mean summer rainfall have potential for inducing prolonged drought conditions, so species able to tolerate prolonged drought conditions may be more prevalent in grasslands overlying shallower soils. Under predicted climate change scenarios, the pattern of rainfall events will change, and the probability of longer drought events will increase (Jenkins, Perry and Prior, 2008; Gohar *et al.*, 2018). These climate changes will operate in tandem with pollutant deposition and inherent geographical and physical attributes of the grassland habitat, with the potential to drive significant change in sensitive habitats. If the resulting patterns of change can be tracked along environmental gradients in existing grasslands, it may help inform steps needed to mitigate for predicted climate and deposition trajectories.

Most climate-plant and deposition-plant interactions are mediated by the medium in which the plant community is rooted, and greater soil depth may offer a degree of buffering from extreme variation in rainfall, temperature and nutrient availability (Fridley, 2002). Soil depth controls soil-moisture and nutrient availability, and influences root architecture and development: put simply, deeper soil offers a larger resource reservoir, though the availability of those resources depends in part on the physical and physiological adaptations by species. Rooting depth is known to vary considerably between species, and deeper rooting depth may confer greater resistance to drought, as roots are able to access soil moisture from further down the soil profile (Castillo *et al.*, 2017). Where the soil is very shallow, the deep-rooting strategy is of little advantage, unless it can adapt to utilise available soil volume rather than soil depth. Shallow roots within the top few centimetres of the soil surface are able to access moisture soon after a rainfall event (von Felten *et al.*, 2012), but may be exposed to a wider range of temperature fluctuations as the insulation provided by overlying soil is reduced. The enhanced cycles of drying and rewetting experienced by shallower soils potentially impose more disturbance to the physical and chemical nature of the soil, the plant community's ability to uptake water and nutrients, than that experienced by deeper soil profiles. Seasonal desiccation of topsoil may contribute to maintaining high species richness (Critchley *et al.*, 2002a), by contributing to habitat heterogeneity.

Spatial variation in soil characteristics are common, and interact with other environmental factors to cascade a number of effects to which the plant community may respond. Soil pH is closely related to the calcium carbonate content of a soil, and in most cases, increases with

depth, i.e. as it gets closer to the weathering front with the parent material. The depth of the weathering front is qualified by the effects of soil depth and soil water flow, but the general trend of lower pH towards the surface has been widely confirmed (Trudgill, 1985; Critchley *et al.*, 2002a; Turner, Baxter and Whitton, 2002). This lowering of pH is due to an accumulation of H^+ ions and the leaching of calcium carbonate from the soil (Hopkins, 2015), and is enhanced in areas with higher rainfall. Rainwater is naturally slightly acidic, and percolation of water will carry acidic products of organic decomposition through a soil profile. The acid nature of rainwater is enhanced by the solution of certain pollutants derived from industrial, agricultural and transport sources, which have potential to cause acidification in the upper layers of even lime-rich calcareous soils.

The main cause of acidification is the deposition of nitrogen oxides (NOx) and ammonia (NH_3) emitted from industrial and agricultural sources. Nitrogen is essential for plant growth and development and is a limiting nutrient for most terrestrial ecosystems. It is, however, possible for an excess of available nitrogen to have a damaging impact on sensitive habitats that have evolved under low-nutrient conditions. An increase in nitrogen deposition has been widely linked to declines in diversity, as a raised nutrient soil status can be exploited by more competitive species to the disadvantage of slower-growing habitat specialists.

Nitrogen emissions are often considered in terms of total N deposition, though there is increasing evidence that the form of nitrogen involved is of importance in determining the effect on sensitive habitats and species (Bobbink *et al.*, 2003; Carroll *et al.*, 2003; Stevens *et al.*, 2011; van den Berg *et al.*, 2016). Oxidised nitrogen (NOx) and reduced nitrogen (NHy) components of total N deposition vary spatially and temporally, depending on source, interactions with climate variables, and how long and far they are carried before deposition. After deposition, NOx and NHy may be further transformed in the soil by chemical (e.g. through temperature or moisture interactions) or biological (via plant roots and soil microbes) processes. NOx is readily soluble in water and preferentially taken up by many plants; NHy (as NH_4^+) can bind to negatively charged clays and organic particulates, removing it from the available pool for nitrification by soil micro-organisms. This results in an accumulation of NHy in the soil, which in turn can lead to acidification of the soil. The two main effects of increased nitrogen deposition are eutrophication, caused by an increase in soil fertility due to increased plant-available nitrogen, and acidification, leading to a lowering of soil pH, and the loss of species unable to tolerate the resulting lower pH conditions. Eutrophication and acidification are not mutually exclusive processes, and it is common for low-nutrient systems to experience both under conditions of enhanced nitrogen deposition.

Calcareous grassland species generally have low nutrient tolerance, so it might be expected that they would be vulnerable to the eutrophying effect of nitrogen deposition. In the UK, however, calcareous grasslands tend to be colimited by nitrogen and phosphorus, as phosphorus solubility is retarded by high soil pH (Morecroft, Sellers and Lee, 1994; Elser *et al.*, 2007). Low phosphorus availability is linked to higher plant diversity, as it advantages slow-growing stress-tolerant species and restricts the growth of competitive species, particularly grasses (Wrage, Chapuis-Lardy and Isselstein, 2010). This does not, however, mean that calcareous grasslands are immune to the effects of increased nitrogen deposition, rather that potential eutrophying effects associated with nitrogen may be subdued. The effects of acidification may be similarly muted, as the high soil pH associated with habitats founded on calcareous substrates are considered less likely to experience high NH_4^+ from atmospheric deposition than has been seen in other habitats, e.g. acid soils (Stevens *et al.*, 2011), due to the buffering effect of high soil pH. Shallower soils may be at greater risk of acidification, as the neutralising of aqueous acids requires a continual replenishment of lime-based material, which may be leached away in areas of high rainfall.

The plant assemblages found on calcareous soils characteristically include species with a preference for high soil pH, low nutrient status, and a tolerance of dry soils. Some species in these communities are strict calcicoles (Jackson, 2000), e.g. *Helictotrichon pratense*, *Scabiosa columbara*, *Hippocrepis comosa*, *Sesleria caerulea* and *Cirsium acaule.* This is one of the most species-rich habitats in Europe (Poschlod and WallisDeVries, 2002), and theoretically a high number of species with different traits should result in a higher degree of complementarity and functional richness, conferring biotic resistance to changing conditions (Byun, de Blois and Brisson, 2013). The presence of individual species, and species richness, are sensitive indicators of perturbations such as nitrogen deposition impacts (Emmett *et al.*, 2011), especially when considered alongside functional characteristics such as mean Ellenberg scores. These provide information about the ecological performance of a species in its realised niche, in terms of the particular levels of water, nutrients, light and acidity required or tolerated by that species (Hill *et al.*, 1999). Ellenberg indicator values (EIVs) are widely used to estimate biotic conditions at a site, and to investigate possible shifts in community composition in the face of variation in those conditions (Bartelheimer and Poschlod, 2016). This, therefore, allows changes in environmental conditions to be monitored over time through the proxy of the character of the vegetation.

Communities on shallower soils may indicate lower Ellenberg R values than those on deeper soils; soil acidification associated with nitrogen, sulphur and acid deposition generally has its

strongest effect in the upper few centimetres (Turner, Baxter and Whitton, 2002), which may involve the whole soil profile where that is itself only a few centimetres deep. Deeper soils are expected to support species with a higher preference for moisture, which would be reflected in a stronger mean site Ellenberg F (moisture) signature. Where communities experiencing higher nitrogen deposition rates exhibit a raised mean community Ellenberg N (nutrient), this would indicate increased nitrogen availability – if associated with deeper soil, this may be simply a response to the greater nutrient resource presented by a greater volume of soil. It may, however, indicate eutrophication, which would be expected to be associated with a corresponding decline in frequency and abundance of low-nutrient specialists in favour of more competitive species (Maskell *et al.*, 2010). Conversely, a lowered value for Ellenberg R with higher deposition rates for nitrogen and sulphur dioxide would indicate a reduction in soil pH and acidification, which would be expected to lead to the decline and eventual loss of species unable to tolerate the lower pH (i.e. calcareous grassland specialists).

As with most other soil characteristics, soil depth can vary at many scales. Under more acid conditions, the weathering of subsoil limestone enhances small-scale variation in topography of the bedrock, and acts to open out existing cracks and fissures through the action of acidic soil water (Trudgill, 1985). If, as suggested above, very shallow soils are likely to experience more acid soil water, then it follows that the rocky substrate will be of a more topographically varied nature. Where a soil is generally shallow, even a small increase in depth has potential to have a proportionately greater effect on soil moisture in terms of greater moisture retention, and on nutrient provision due simply to the (slightly) greater soil volume. Diversity in these species-rich habitats is driven by the fact that each species' requirements are all very slightly different, so the provision of multiple small-scale microhabitats should lead to an increase in species richness, in contrast to the unifying effect of deeper soil, where differences in soil depth become less critical to plants in a particular locale. This has been seen in a long-running field study at Buxton, where individual species were found to show particular soil depth affinities (Fridley *et al.*, 2011). Moreover, in the course of the 15-year study, the community was found to undergo reorganisation at the sub-plot scale in response to the imposed climate change treatments.

The potential protection afforded by deeper soil may serve to reduce microhabitat diversity, through the removal of the more restricted (and therefore specialised) niches, and thus reduce species richness through the replacement of species adapted to those niches and the particular stresses they involve, with more competitive species. It is hypothesised that grassland communities in shallower soils will be more species-rich (H4.1) and diverse (H4.2), as will those

where soil depth is more varied (H4.3), due to greater provision of micro-habitats, and a reduced opportunity for more competitive species to dominate. Communities on shallower soils are also expected to present stronger responses to variation in certain environmental variables such as rainfall and temperature, under the expectation that shallower soils will dry out sooner under drought conditions, and suffer a greater thermal range both diurnally and seasonally. This would be expected to lead to a greater proportion of stress-tolerant and ruderal species in such communities (H4.4). Where grassland communities receive higher summer rainfall, or have higher nutrient availability, they might be expected to include a greater proportion of competitive species, to the disadvantage of the calcareous grassland specialists (H4.5).

This study is intended to investigate variation in functional characteristics of calcareous grasslands along a number of environmental gradients in the UK. In particular, it is intended to investigate potential mediation provided by soil depth, to variation in climate and pollutant deposition, and to assess the extent to which greater soil depth buffers plant communities against low rainfall, eutrophication and acidification. This will be investigated via community species richness responses to the interaction of environmental variables such as rainfall and nitrogen deposition with varying soil depth (H4.6). It is proposed that species richness will decline with increasing soil depth, as habitat specialists are replaced by generalists, and that there will be a higher competitive element in the community C-S-R signatures of sites with greater mean soil depth. It is therefore expected that communities on shallower soils will have a higher stress-tolerant element in site C-S-R signatures. Evidence of eutrophication and acidification will be looked for, using the plant communities' Ellenberg indicator values to indicate habitat conditions with reference to soil depth, climate and deposition variables.

Hypotheses tested:

H4.1: Plant species richness will decline with increasing soil depth.

H4.2: Plant communities will be more diverse on shallower soils.

H4.3: Greater heterogeneity in soil depth will result in an increase in species richness.

H4.4: Plant communities on shallower soils will have a higher stress-tolerant element in site C-S-R signatures.

H4.5: Plant communities on sites with greater nutrient availability will contain a greater proportion of competitive species (greater nutrient availability may be due to a number of factors, including higher deposition rates of atmospheric nitrogen, deeper soil or higher rainfall).

H4.6: Soil depth will act to modify the response of species richness to other environmental variables.

4.2 Method

4.2.1 Site selection

Natural England's designated sites database was interrogated for sites that fulfilled the following criteria, and a subset of sites picked that provide a broad latitudinal spread across England (Figure 4.1), from Crook Peak in Somerset in the south, to the northernmost site at Thrislington, County Durham (Table 4.1).

Criteria:

- Sites were to be within an SSSI, on calcareous grassland designated as being in favourable condition, to ensure surveys were undertaken on good quality calcareous grassland.

- In order to reduce possible confounding factors due to variations in underlying solid geology, sites were restricted to "hard" limestone.

- As there is a high degree of variation in environmental conditions between north- and south-facing sites, the surveys were all carried out on generally south-facing slopes.

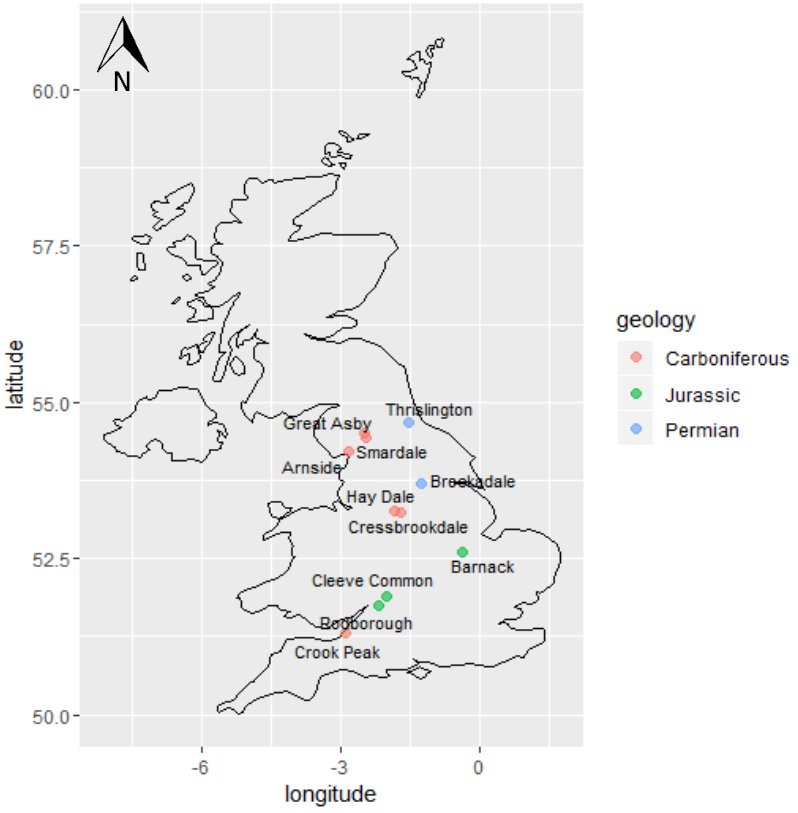


Figure 4.1 Site locations.

Table 4.1 Site list: underlying geology; latitude and longitude for one reference quadrat per site; elevation given is mean elevation (metres above ordnance datum) for survey quadrats. Abb. gives the abbreviated site name, where it has been used in figures and tables.

site	abb.	geological period	latitude	longitude	elevation
Arnside Knott	arn	Carboniferous	54.19242	-2.83504	125.85
Barnack Hills & Holes	bar	Jurassic	52.62833	-0.41402	46.22
Brockadale	bro	Permian	53.64902	-1.22846	27.08
Cleeve Common	clv	Jurassic	51.92962	-2.01402	298.56
Cressbrookdale	crs	Carboniferous	53.27074	-1.74243	216.00
Crook Peak	crk	Carboniferous	51.29154	-2.86871	71.50
Great Asby Scar	gta	Carboniferous	54.47887	-2.51924	360.86
Hay Dale	hay	Carboniferous	53.28986	-1.82056	293.70
Rodborough Common	rod	Jurassic	51.73188	-2.22212	177.00
Smardale Gill	sma	Carboniferous	54.45232	-2.42769	239.40
Thrislington	thr	Permian	54.68882	-1.50641	130.50

4.2.2 Vegetation

Desk-based assessment was used to determine initial target areas. This included consultation with Natural England and owner/occupiers, and scrutiny of available maps and other records. Sites were visited in May 2018, and individual surveys were completed in one day. A representative sample of the vegetation and soil depth on each site was made using randomly placed quadrats, recording the presence of species rooted within a 15 cm x 15 cm area. Five soil depth measurement were taken for each quadrat – one at each corner, and one in the centre, to ensure that species present could be closely tied to soil depth. As plant roots are strongly geotropic, soil depth was measured vertically rather than perpendicular to the surface, which was often sloping. Twenty quadrats were surveyed per site.

Species identification follows Rose and O'Reilly (2006) and Stace (2010) for forbs, legumes, sedges and woody species; grasses follow Hubbard (1984). Ellenberg indicator values were taken from Hill *et al.*, (1999). Life history data were taken from BRC Atlas of British and Irish Flora (https://www.brc.ac.uk/plantatlas/). C-S-R strategy data were taken from the UCPE online tool developed by Sheffield University (Hunt *et al.*, 2004); for species not included in UCPE database, other sources were used for C-S-R information, namely Pierce *et al. (*2017) for *Bromus commutatus*, *Vicia sativa, Saxifraga stellaris, Sesleria caerulea* and *Thymus pulegoides. Galium pumilum* and *Astragalus danicus* use the strategies allocated by Riibak *et al.* (2015), and *Trifolium scabrum* was assigned as per the allocation of Navas *et al.* (2010).

Species were assigned to plant groups – graminoids (grasses, sedges and wood-rush species), legumes, non-leguminous forbs (hereafter, referred to as "forbs"), and woody species. Species' abundance was estimated from presence-absence frequency in survey quadrats.

4.2.3 Environmental factors

Environmental and meteorological data for each site were gathered from a number of sources, and are summarised in Table 4.2 . Definitions of variables are as used by the source authority:

- Information on solid geology from Natural England's designated sites database, as all locations were within SSSIs (see https://designatedsites.naturalengland.org.uk/).

- Mean annual rainfall and temperature are given as the three-year average for 2015-2017. Data for 2015 and 2016 were acquired from the UKCP09 5 km gridded data set available at http://data.ceda.ac.uk/badc/ukcp09/data/gridded-land-obs/gridded-land-obs-monthly/ (Met Office, Hollis and McCarthy, 2017). Data for 2017 were downloaded from the HadUK-Grid_grid 1 km gridded climate observations available at https://catalogue.ceda.ac.uk/uuid/2a62652a4fe6412693123dd6328f6dc8 (Met Office *et al.*, 2018)

- Mean summer temperature and rainfall were derived from Met Office climate data for 2015-2017 (as above), averaged over June to August for those years.

- Nitrogen deposition (kg ha^{-1} yr^{-1}), nitrogen oxides (as NO_2) concentration, ammonia concentrations (µg NH_3 m^{-3}) and critical loads for N were all sourced from the Air Pollution Information Service (APIS) website http://www.apis.ac.uk/search-location. Metric used was the three-year average for 2015-2017 data.

- Sulphur dioxide (µg m^{-3}) and acid deposition (keq ha^{-1} yr^{-1}) were sourced from the Air Pollution Information Service, using 2015-2017 three-year averages. Acid deposition is given as total acidifying deposition, calculated as: acid deposition = (S + (NOx + NHy)) – NMBC (non-marine base cations, Ca + MG) where S is non-marine sulphur wet + dry deposition, NOx and NHy are both wet + dry deposition, and NMBC is wet deposition. Further details in NEGTAP (2001).

- Slope and aspect were noted during site visits; elevation data were derived from GPS output during site visits (GPSMAP 64S, Garmin (Europe) Ltd., Southampton).

- Mean site soil depth was calculated from the 100 soil depth measurements taken across the 20 quadrats at each site.

Table 4.2 Environmental factors considered for each survey location; all climate and pollutant variables are the three-year average for 2015-2017. Units are as follows: annual and summer temperature (°C) and rainfall (mm). Total N is kg N ha^{-1} yr^{-1}; NOx concentration is µg NOx (as NO_2) m^{-3}; NHy concentration (as NH_3) in µg m^{-3}; SO2 is in µg m^{-3}. Critical load is in kg N ha^{-1} yr^{-11}, as are exceedance values for the lower and upper limits, i.e. the amount that total annual N deposition exceeds the lower and upper limits of the current critical load for nitrogen on calcareous grasslands.. Mean soil depth is given in cm. All data sources as listed above. Site names are abbreviated as per Table 4.1.

site	mean annual temp	mean annual rain	mean summer temp	mean summer rain	total N	NOx	NHy	NOx:NHy ratio	critical load min	critical load max	exceeds critical load lower limit	exceeds critical load upper limit	mean soil depth
arn	9.35	1241.97	15.07	104.98	22.26	12.15	2.62	4.64	15	25	7.26	-2.74	4.22
bar	10.75	539.03	16.73	49.24	17.64	12.7	1.95	6.51	15	25	2.64	-7.36	8.08
bro	10.46	608.53	15.91	67.98	18.62	7.03	1.19	5.91	15	25	3.62	-6.38	12.37
clv	9.77	790.56	16.20	62.96	17.22	10.75	2.26	4.76	15	25	2.22	-7.78	8.42
crk	10.99	788.02	16.45	68.87	18.76	7.8	2.58	3.02	15	25	3.76	-6.24	6.08
crs	9.29	985.39	14.75	82.73	33.46	9.82	2.77	3.55	15	25	18.46	8.46	10.24
gta	7.91	1851.40	12.81	137.99	15.4	4.53	0.98	4.62	15	25	0.4	-9.6	8.15
hay	8.98	1254.09	14.60	86.50	28.7	8.54	2.4	3.56	15	25	13.7	3.7	9.82
rod	10.84	782.74	16.16	60.54	16.1	16.66	2.07	8.05	15	25	1.1	-8.9	8.09
sma	8.80	1580.67	13.41	115.37	14.98	4.82	1.02	4.73	15	25	-0.02	-10.02	4.72
thr	9.52	614.95	14.62	63.41	20.86	13.26	2.81	4.72	15	25	5.86	-4.14	11.41

4.2.4 Analysis

For each site, the following metrics were calculated:

- species richness (SR), being the count of all species of vascular plant and bryophyte present.

- Simpson's Index of Diversity (SID) for infinite populations (equation as per that used in Chapter 2), using the *diversity(simpson)* function in the R package *vegan* (Oksanen, 2018).

- site C-S-R signatures, using the online tool associated with Hunt *et al.* (2004). This is a quantitative tool that uses plant functional types (in the context of Grime's C-S-R classification system) to derive a community signature that represents the balance of the C, S and R strategy elements within the community.

- frequency-weighted site means for Ellenberg Indicator Values (EIV) for nutrients (N), moisture(F) and reaction (R) using the equation: $\sum(x_i \,.\, A_i)/(\sum A_i)$, where x_i = EIV of species i, and A_i = frequency, i.e. number of quadrats in which species *i* was observed (Klaus *et al.*, 2012).

Pairwise correlations between environmental and community variables (species richness, Simpson's Index of Diversity, etc.) were carried out using Spearman's rank correlation, as many distributions were not normally distributed; Spearman's rank correlation determines the degree to which a relationship between two variables is monotonic. The data were highly variable across the eleven sites, and Spearman's correlation was considered more appropriate than other methods as it is less sensitive to outliers than Pearson's correlation, which is a potential issue with a relatively small number of sites.

Generalised linear mixed effects models were used to explore the relationships between quadrat species richness and quadrat-level soil depth variables, using the *glmer* function of the *lme4* package in R (Bates *et al*., 2015). A Poisson error distribution was used with a log link function, and 'site' was included as a random effect, to account for unrecorded variation by site, and to avoid pseudoreplication due to nestedness of quadrats within each site. The outline model structure was glmer(*quadrat species richness ~ soil depth metric + (1|site)*), where *site* was a random factor with eleven levels. The relationship between quadrat-level soil depth

metrics and proportions of Grime C, S and R in quadrat-level Grime C-S-R signatures were explored using linear mixed effect models with site as a random factor.

Generalised linear models were used to explore the relationships between site-level species richness and diversity measure (Simpson's Index of Diversity) and environmental factors including soil depth metrics. For site-level species richness, a Poisson variance distribution was used with a log link function, using the *glm*() function in base R. Where species richness data were found to be over- or under-dispersed, generalised linear models with a quasipoisson variance distribution were used. Simpson's Index of Diversity can be considered a measure of average of species frequency; in this analysis, Simpson's Index of Diversity was treated as a proportion (bounded by 0 and 1), and investigated via generalised linear models using a binomial distribution with a logit link function. Where data were found to be overdispersed (i.e. where residual deviance was substantially less than the residual degrees of freedom), a quasibinomial distribution was used in the models. Proportion of competitive or stress-tolerant elements in site C-S-R signature was investigated using generalised linear models; although C-S-R signatures comprise proportion data with three components internally constrained to 1, it was felt that this constraint could be ignored by investigating the C, S and R component proportions individually.

Non-metric multidimensional scaling ordination (NMDS) was carried out on species data, using the *metaMDS()* function in R package *vegan* (Oksanen, 2018), to assess which (if any) of the environmental variables had a significant association with community composition. Convergence was achieved with two dimensions (k = 2), with a stress level of 0.1385; Bray Curtis was selected as the most appropriate method of calculating dissimilarity between communities as it handles species' absence well; Wisconsin double standardisation was applied to the dissimilarity indices; permutations were based on multiple random starts with centring and rotation of the resulting configuration using Procrustes rotation to derive the best fit within ordination space. Environmental variables were selected for inclusion in the ordination through consideration of degree of correlation with diversity metrics and collinearity with other environmental variables, and as a matter of making ecological sense of the communities. The variables that were selected were latitude, longitude, elevation, mean site soil depth, mean annual rainfall, mean annual temperature, total N, NOx and NHy deposition, and NOx:NHy ratio.

4.3 Results

Results pertinent to individual hypotheses can be found as follows:

- H4.1 Plant species richness will decline with increasing soil depth *in section* 4.3.2
- H4.2 Plant communities will be more diverse on shallower soils *in section* 4.3.3
- H4.3 Greater soil depth heterogeneity will result in increased species richness *in section* 4.3.2
- H4.4 Plant communities on shallower soils will have a higher stress-tolerant element in site C-S-R signatures *in section* 4.3.7.2
- H4.5 Plant communities will have a higher competitive element in the C-S-R signature in association with a) higher rainfall, b) higher deposition rates of atmospheric nitrogen, or c) deeper soil *in section* 4.3.7.2
- H4.6 Soil depth will act to modify the community species richness response to other environmental variables *in section* 4.3.6

4.3.1 Summary descriptive analysis

4.3.1.1 Soil metrics

Soil depth metrics were collected at quadrat level, which were then summarised for site-level analysis (Figure 4.2), and ranged from 1 cm to 35 cm.

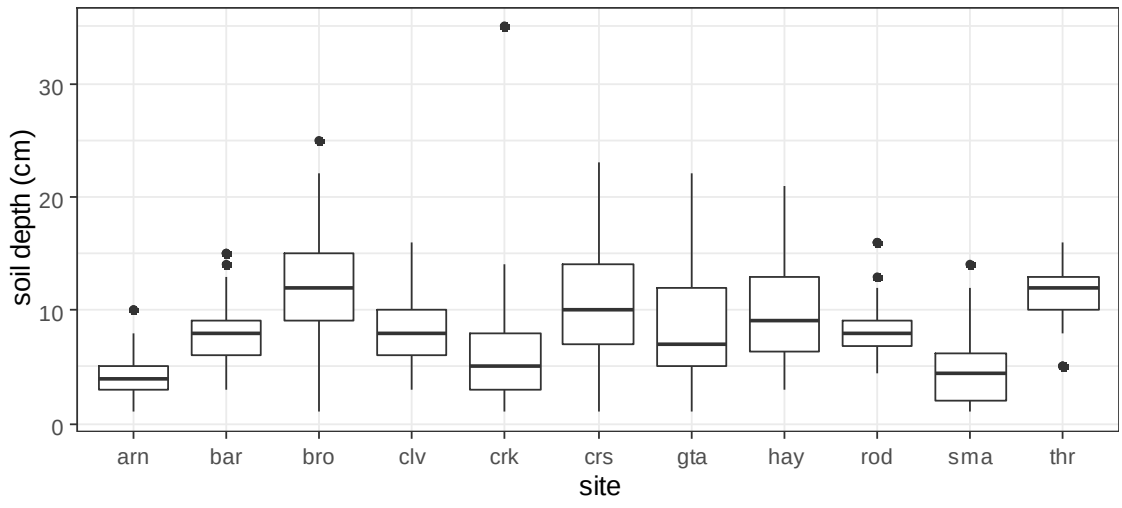


Figure 4.2 Soil depth across all sites. Bars on boxplots indicate site median soil depth; points are individual depth measurements. For each site, n = 100. Site names are abbreviated as per Table 4.1.

Mean quadrat soil depth ranged from 1.4 cm to 18.4 cm. Mean site soil depths ranged from 4.22 cm at Arnside Knott to 12.37 cm at Brockadale. The lowest minimum soil depth was 1 cm; the highest maximum soil depth recorded was 35 cm (Crook Peak) (Table 4.3), though this was atypical and may represent a burrow, root disturbance, solution hole or other subsoil/substrate feature. The majority of soil depth measurements were less than 8 cm (617 of 1100 measurements, i.e. 56%), including 305 that were 5 cm or less; 384 depth measurements (35%) were 10 cm or deeper.

Soil depth heterogeneity ("range") was calculated as the difference between the maximum and minimum soil depths measured at each scale unit, i.e. at quadrat and site levels.

Table 4.3 Summary site-level soil depth data. All measurements are in cm.

site	minimum	maximum	range	mean	SD
Arnside	1	10	9.0	4.22	1.71
Barnack	3	15	12.0	8.08	2.30
Brockadale	1	25	24.0	12.37	4.48
Cleeve Common	3	16	13.0	8.42	3.11
Crook Peak	1	35	34.0	6.08	4.15
Cressbrookdale	1	23	22.0	10.24	4.42
Great Asby	1	22	21.0	8.15	4.94
Hay Dale	3	21	18.0	9.82	4.15
Rodborough	4.5	16	11.5	8.09	2.28
Smardale	1	14	13.0	4.72	2.88
Thrislington	5	16	11.0	11.41	2.11

4.3.1.2 Environmental variables

Environmental variables (rainfall, temperature, and all atmospheric deposition variables) were collected from the nearest available meteorological station to each site. Temperature and rainfall values reflected national weather patterns, with mean temperature increasing to the south and east, and rainfall values increasing towards the north and west. There were few significant relationships between the environmental pollutants considered and climatic variables, other than NOx, which was found to significantly increase as mean summer rainfall decreased (Spearman's *rho* = -0.727, n = 11, p = 0.015). Sulphur dioxide (SO_2) and nitrogen showed similar patterns of deposition, increasing with increasing temperature, and decreasing with increasing rainfall. Acid deposition rates were strongly associated with total nitrogen (Spearman's *rho* = 0.855, n = 11, p = 0.002) and NHy (Spearman's *rho* = 0.645, n = 11, p = 0.037). See Table 4.2 above for values of environmental variables for each site.

4.3.1.3 Species composition

A total of 111 species were observed in survey quadrats across all sites. Of these, 28 were grasses, 69 forb species, eleven legumes and three woody species (all *Crataegus*, apart from one incidence each of *Vaccinium myrtillus* and *Calluna vulgaris*, both from Great Asby).

Over half (n = 61) the observed species were rare, in that they occurred in fewer than 5% of surveyed quadrats. Thirty-three species were present in fewer than 1% of quadrats, i.e. in either one or two quadrats only (Figure 4.3). Only three species were found in more than 50% of quadrats: *Sanguisorba minor* (59%), *Carex flacca* (66%) and *Lotus corniculatus* (68%).

The ten most-recorded species were all perennials; five were graminoids (*Briza media* (present in 30% of quadrats), *Bromus erecta* (40%), *Anthoxanthum odoratum* (47%); *Festuca ovina* (47%), *Carex flacca* (66%)); one legume. *Lotus corniculatus* (68%); and four forbs (*Hieracium pilosella* (32%), *Helianthemum nummularium* (42%), *Plantago lanceolata* (48%), *Sanguisorba minor* (59%)).

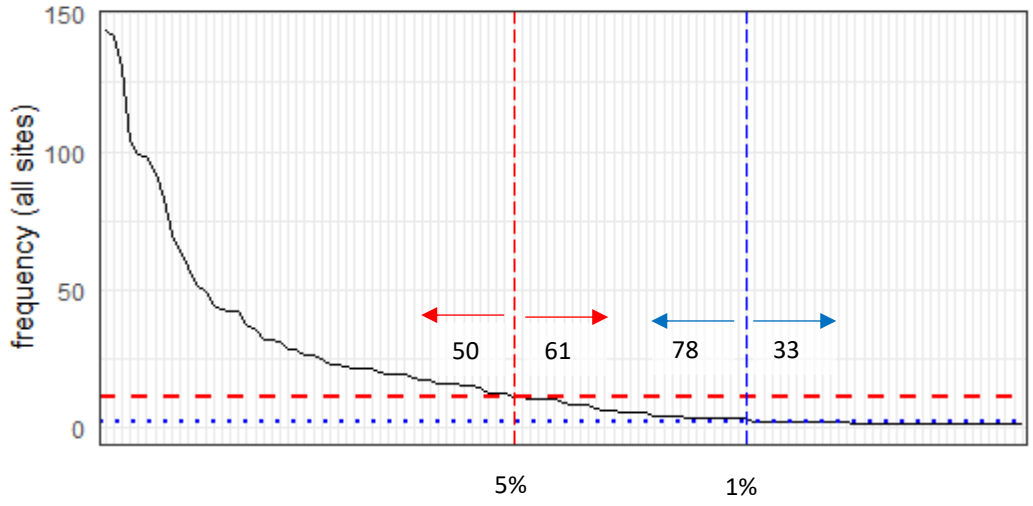


Figure 4.3 Species frequency curve for the calcareous grassland habitat as surveyed. Frequency is the total number of quadrats in which a particular species was observed, across all sites. Horizontal lines indicate abundance at 5% (red, dashed) and 1% (blue, dotted); vertical lines indicate intercepts with species abundance curve.

Two species (*Astragalus danicus* and *Galium pumilum*) are vulnerable IUCN Red Data List species, and considered nationally endangered (Cheffings *et al.*, 2005); *Arabis hirsuta* has near-threatened status in the UK.

4.3.1.4 *Species richness and diversity*

Species richness was measured at site level and at quadrat level (Figure 4.4). Species richness varied between sites, with the lowest species count at Arnside Knott (species richness = 25) and the highest number of species recorded at Brockadale and Crook Peak (both species richness = 46); species richness was also assessed within plant groups (Table 4.4).

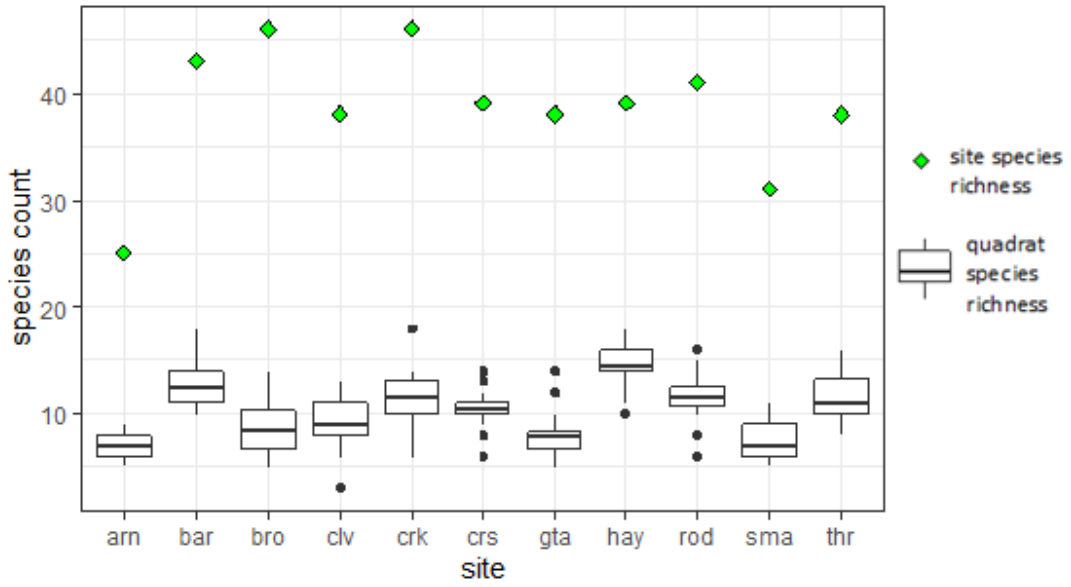


Figure 4.4 Summary of species richness data at site and quadrat level. Quadrat species richness = number of species in an individual quadrat; site species richness is the number of discrete species observed within the 20 quadrats surveyed on a site.

Table 4.4 Species richness (SR) metrics. "site SR" is the total number of different species encountered on each site; "mean quadrat SR" is the mean number of species encountered in individual quadrats on a particular site.

site	site SR	grasses	forbs	legumes	woody	mean quadrat SR
Arnside Knott	25	9	13	3	0	4.23
Barnack	43	12	27	4	0	8.08
Brockadale	46	13	27	5	0	12.37
Cleeve Common	38	17	18	2	1	8.42
Cressbrookdale	39	16	19	3	1	6.08
Crook Peak	46	15	24	6	1	10.24
Great Asby Scar	38	14	20	2	2	8.15
Hay Dale	39	13	22	3	1	9.82
Rodborough	41	15	23	3	0	8.09
Smardale Gill	31	15	13	2	1	4.72
Thrislington	38	12	22	4	0	11.41

Quadrat species richness ranged from three species (one quadrat at Cleeve Common) to 18 species (one quadrat at each of Barnack Hills and Holes, Crook Peak and Hay Dale.

Simpson's Index of Diversity was calculated for each site, so was only assessed against environmental and soil depth metrics at site level. Simpson's Index of Diversity for all sites was high, and ranged from 0.85 at Arnside Knott to 0.93 in Hay Dale (Figure 4.5) (higher values of this diversity index indicate higher diversity). Simpson's Index of Diversity was significantly associated with minimum soil depth (Spearman's *rho* = 0.71, r^2 = 0.50, n = 11, p = 0.015), and weakly associated with mean summer rainfall (Table 4.5).

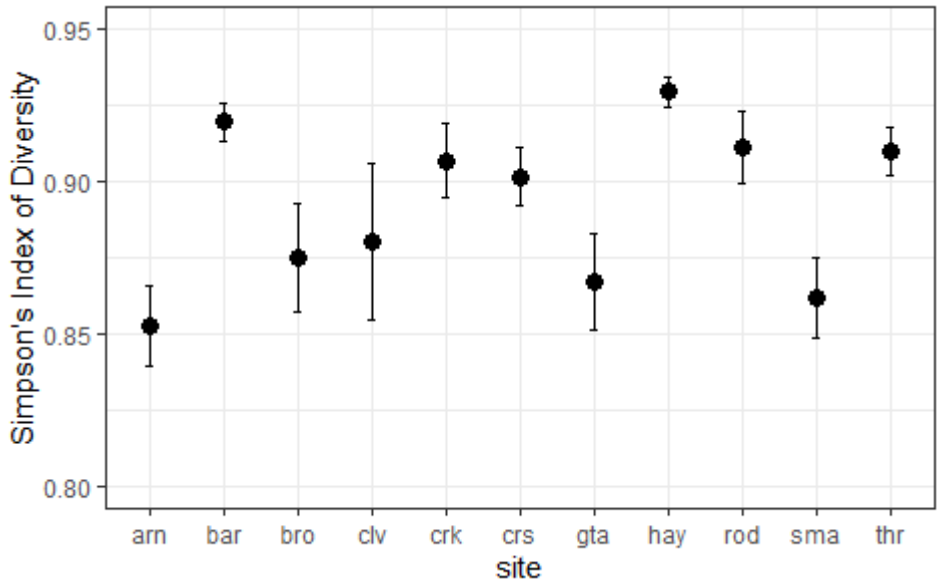


Figure 4.5 Simpson's Index of Diversity for all sites. Site names are abbreviated as per Table 4.1.

4.3.1.5 Correlation between soil and environmental variables and site-level diversity metrics

Correlation coefficients between environmental variables and site-level diversity metrics (species richness and Simpson's Index of Diversity) are given in Table 4.5, below.

*Table 4.5 Correlation between site-level diversity metrics (species richness and Simpson's Index of Diversity) and environmental variables. Table shows Spearman's rho (r) for these diversity metrics and environmental and community factors. Coefficients were calculated using three-year averages 2015-2017. Significance levels are indicated as follows: * p<0.05, ** p<0.01, *** p<0.001. For all correlations, n = 11. Effect sizes indicated by the modulus of rho are taken to be as follows: rho 0.00-0.19 very weak, 0.20-0.39 weak, 0.40-0.59 moderate, 0.60-0.79 strong, 0.80-1.00 very strong.*

environmental variable	**site species richness**			**Simpson's Index of Diversity**		
	rho	**r^2**	**p**	***rho***	**r^2**	**p**
latitude	-0.613	0.376	0.045 *	-0.436	0.190	0.183
longitude	0.350	0.123	0.291	0.473	0.224	0.146
elevation	-0.553	0.306	0.078	-0.118	0.014	0.734
slope	0.084	0.007	0.806	-0.262	0.069	0.436
mean site soil depth	0.318	0.101	0.341	0.309	0.095	0.356
minimum soil depth	0.048	0.002	0.888	0.710	0.504	0.015 *
maximum soil depth	0.674	0.454	0.029 *	0.193	0.037	0.570
soil depth range	0.577	0.333	0.063	-0.014	<0.001	0.968
annual rainfall	-0.641	0.411	0.034 *	-0.463	0.214	0.154
annual temperature	0.691	0.477	0.019 *	0.409	0.167	0.214
summer rainfall	-0.525	0.276	0.097	-0.609	0.371	0.052
summer temperature	0.585	0.342	0.059	0.336	0.113	0.313
total N deposition	0.120	0.014	0.726	0.282	0.080	0.402
NOx	0.018	<0.001	0.957	0.491	0.241	0.129
NHy	-0.083	0.007	0.808	0.255	0.065	0.451
Nox:NHy ratio	0.098	0.604	0.777	0.091	0.008	0.797
sulphur dioxide	-0.018	<0.001	0.957	0.327	0.107	0.327
acid deposition	-0.138	0.019	0.685	-0.073	0.005	0.839

4.3.2 Influence of soil depth metrics on species richness

Soil depth metrics considered as potential influencers of species richness were minimum soil depth, maximum soil depth, mean soil depth; soil depth range and the standard deviation of soil depth were also investigated, as possible reflectors of soil depth heterogeneity.

Associated hypotheses:

H4.1 Plant species richness will decline with increasing soil depth

H4.3 Greater soil depth heterogeneity will result in increased species richness

4.3.2.1 Site level

Of the soil depth metrics, maximum soil depth accounted for the most variance in site species richness (Spearman's *rho* = 0.67, r^2 = 0.45, n = 11, p = 0.03), and site soil depth range had a moderate effect on species richness (Spearman's *rho* = 0.58, r^2 = 0.33, n = 11, p = 0.06) (Figure 4.6).

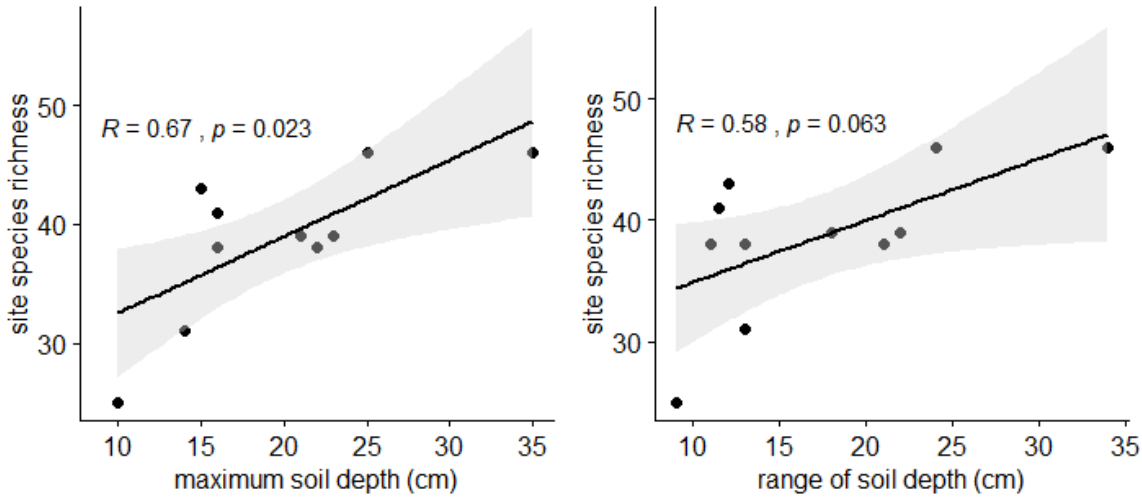


Figure 4.6 Regression of site species richness against maximum soil depth and range of soil depth on site.

The increase in site species richness with increasing maximum soil depth suggested that there were more species on sites with even only occasionally much deeper microsites.

This apparent association was confirmed by generalised linear models, which indicated that site species richness was significantly associated with maximum soil depth (t = 2.898, 10 df, p = 0.2)

and soil depth range (t = 2.280, 10 df, p = 0.05), and that mean soil depth across sites was only weakly associated with site species richness (Table 4.6).

Table 4.6 Output from generalised linear models investigating the influence of soil depth metrics on site species richness. For all models, null degrees of freedom = 10.

***model*: site species richness ~ soil depth metric**					**confidence interval**	
metric	**estimate**	**SE**	**t-value**	**p**	**2.5%**	**97.5%**
mean	0.0369	0.0176	2.095	0.0657	0.0025	0.0716
maximum	0.0160	0.0055	2.898	0.0177	0.0051	0.0267
minimum	0.0165	0.0342	0.481	0.6420	-0.0513	0.0828
range	0.0126	0.0055	2.280	0.0486	0.0016	0.0233
SD	0.0558	0.0462	1.209	0.2270	-0.0350	0.1460

4.3.2.2 Quadrat level

There was great variation in both the direction and magnitude of the relationship between species richness and soil depth metrics at quadrat level (Table 4.7, Figure 4.7).

Table 4.7 Spearman rank correlation coefficients (rho) and probability scores (p) for quadrat species counts and quadrat-level soil depth metrics (all depth measurements in cm). For all cases, n = 20.

	mean quadrat soil depth		**maximum quadrat soil depth**		**minimum quadrat soil depth**		**quadrat soil depth range**	
site	***rho***	**p**	***rho***	**p**	***rho***	**p**	***rho***	**p**
arn	0.20	0.39	0.13	0.57	0.31	0.18	-0.23	0.33
bar	-0.07	0.78	0.02	0.95	-0.11	0.63	0.32	0.17
bro	-0.25	0.29	-0.22	0.36	-0.39	0.10	-0.03	0.90
clv	-0.13	0.58	-0.20	0.40	-0.04	0.86	-0.51	0.02
crk	0.49	0.03	0.46	0.04	0.52	0.02	-0.07	0.76
crs	-0.11	0.65	-0.23	0.32	-0.06	0.80	-0.29	0.21
gta	0.24	0.30	0.28	0.34	0.07	0.76	0.37	0.11
hay	0.33	0.16	0.17	0.47	0.35	0.13	-0.09	0.72
rod	0.44	0.05	0.46	0.04	-0.43	0.06	-0.33	0.15
sma	0.15	0.54	0.10	0.69	0.18	0.44	-0.05	0.84
thr	0.35	0.13	0.20	0.39	0.26	0.27	0.09	0.72

The influence of soil metrics on quadrat species richness was investigated via generalised mixed effect models, where site identity was included as a random effect in order to account for variation in unmeasured aspects of each site (specified in R as: glm(quadrat species richness ~

soil depth metric + (1|site))). These models indicated that there was no overarching linear relationship between the number of species observed in a quadrat, and the soil metrics associated with that quadrat, when all data were pooled (Table 4.8).

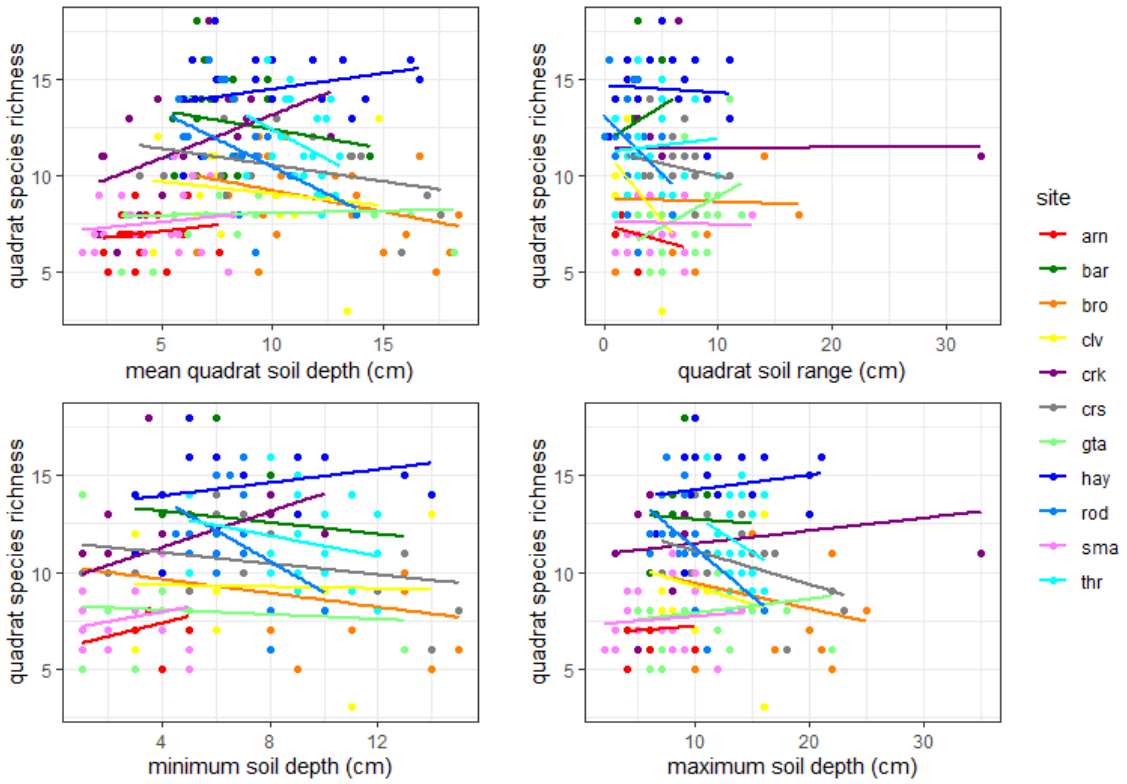


Figure 4.7 Linear regression plots for quadrat species richness and quadrat-level soil depth metrics.

Table 4.8 Output from generalised linear mixed effect models investigating the influence of soil depth metrics on quadrat species richness. For all models, residual deviance was calculated on 217 df.

model: quadrat species richness ~ soil depth metric + (1|site)					**confidence interval**	
metric	**estimate**	**SE**	**t-value**	**p**	**2.5%**	**97.5%**
mean	-0.0010	0.0074	-0.128	0.898	-0.0157	0.0135
maximum	-0.0017	0.0055	-0.310	0.757	-0.0127	0.0090
minimum	-0.0006	0.0077	-0.075	0.940	-0.0157	0.0144
range	-0.0021	0.0067	-0.309	0.757	-0.0156	0.0107
SD	-0.0067	0.0162	-0.413	0.680	-0.0394	0.0241

The response of quadrat species richness to soil metrics varied from site to site (Figure 4.8, Figure 4.9, Figure 4.10 and Figure 4.11); quadrat species richness increased with increasing mean quadrat soil depth at Arnside, Crook Peak, Great Asby, Hay Dale and Smardale, and declined with increasing mean quadrat soil depth at all other sites. There were more species

observed in quadrats with a wider range of soil depths at Barnack, Crook Peak, Great Asby and Thrislington.

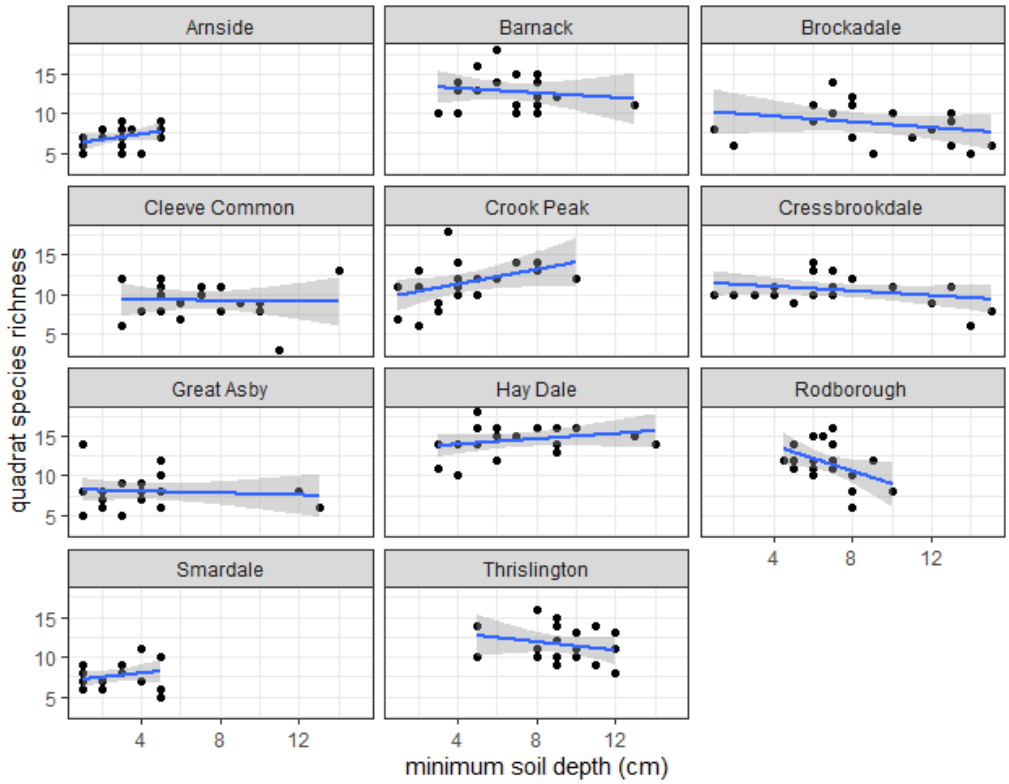


Figure 4.8 Regression of quadrat species richness against minimum quadrat soil depth.

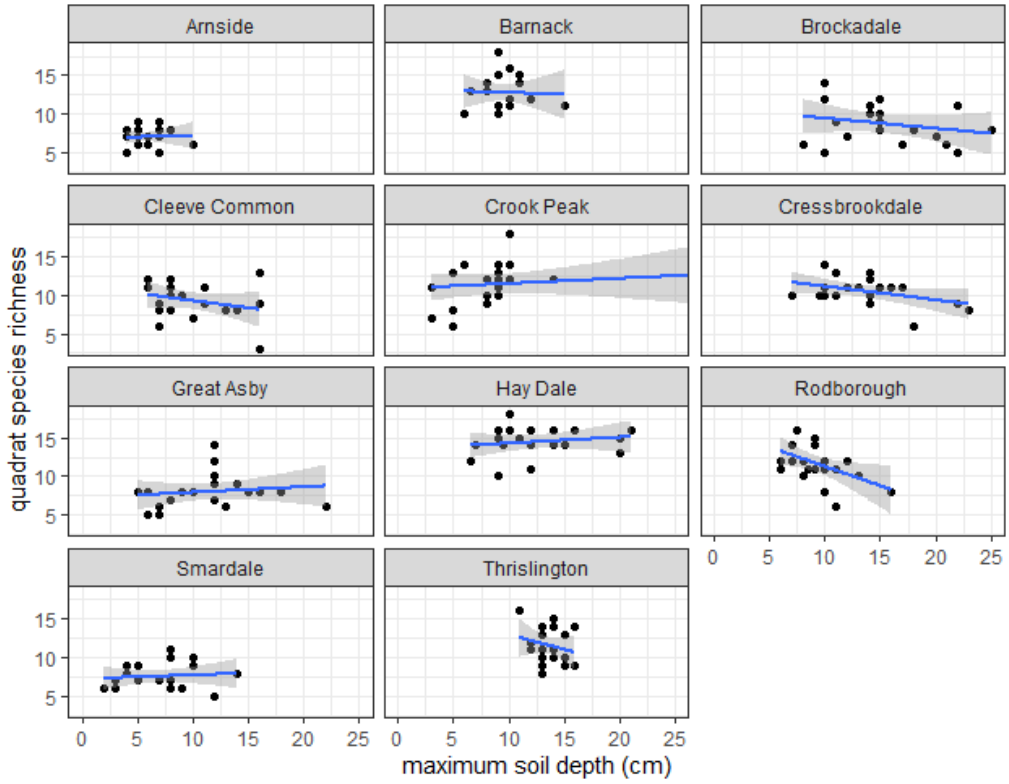


Figure 4.9 Regression of quadrat species richness against maximum quadrat soil depth. Crook Peak regression extends to outlier with maximum soil depth of 35 cm; this has been omitted to aid visualisation.

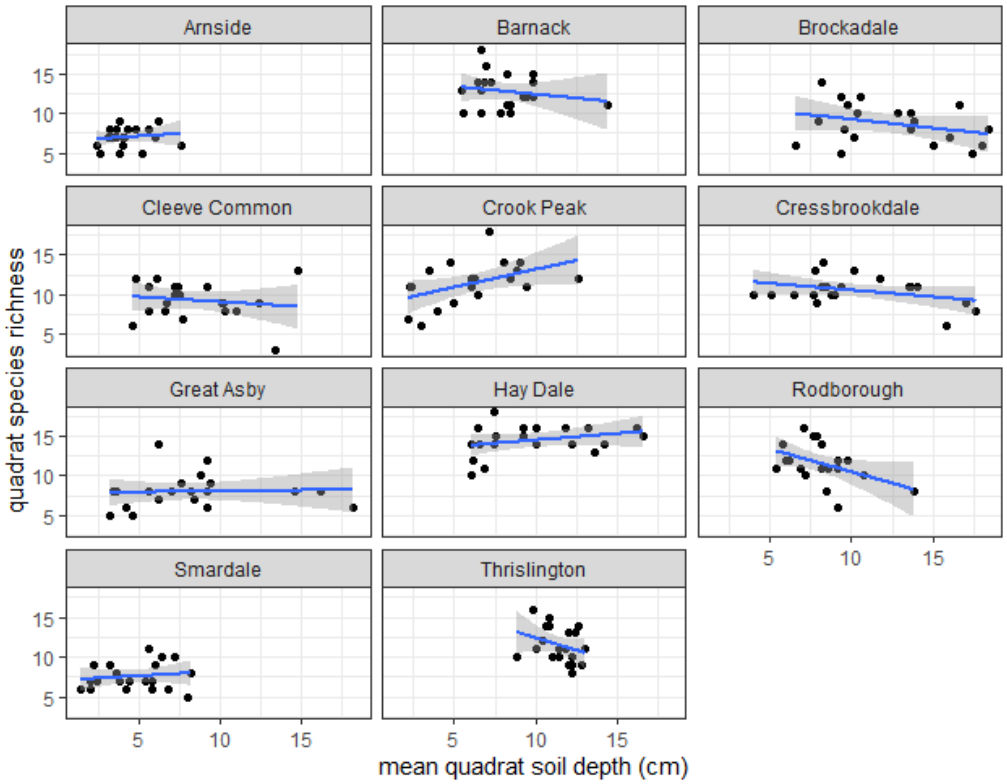


Figure 4.10 Regression of quadrat species richness against mean quadrat soil depth.

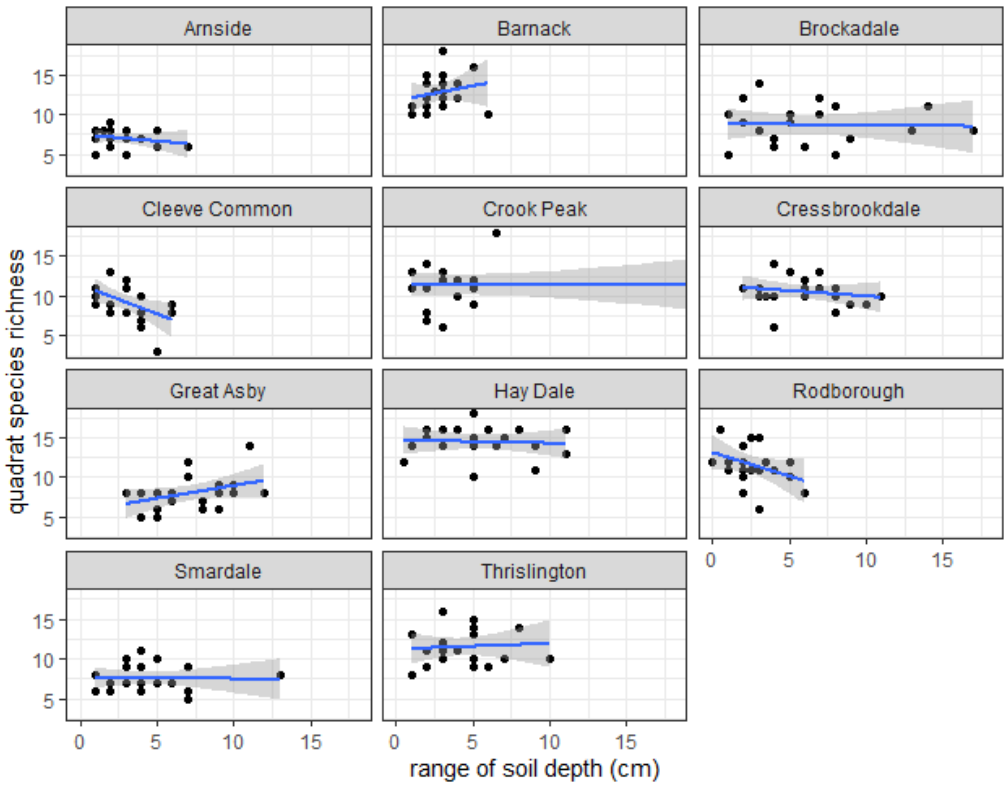


Figure 4.11 Regression of quadrat species richness against range of soil depth per quadrat. Crook Peak regression extends to an outlier with soil depth range of 34 cm; this has been omitted to aid visualisation

Within-site relationships of soil depth metrics with quadrat-level species richness were investigated via generalised linear models using a poisson variance distribution, and few significant relationships were found between the number of species observed in a quadrat and the soil metrics associated with that quadrat (Table 4.9). The models indicated that the relationship between quadrat species richness and mean quadrat soil depth was significant at Crook Peak (t = 2.140, 18 df, p = 0.046) and Rodborough (t = -0.052, 18 df, p = 0.046). Quadrat species richness at Rodborough was significantly reduced in quadrats with higher maximum quadrat soil depth (t = -2.345, 18 df, p = 0.031). At Cleeve Common, quadrat species richness declined significantly with increasing range of soil depth within quadrats (t = -0.082, 18 df, p = 0.027).

Table 4.9 Model output from generalised linear models for quadrat soil metrics influence on quadrat species richness. For all models, residual deviance was calculated on 18 df.

***model*:** quadrat species richness ~ minimum soil depth + (1|site)				
site	**estimate**	**SE**	**t-value**	**p**
Arnside	0.0498	0.0320	1.558	0.137
Barnack	-0.0117	0.0182	-0.644	0.528
Brockadale	-0.0202	0.0171	-1.185	0.251
Cleeve Common	-0.0025	0.0211	-0.118	0.907
Crook Peak	0.0392	0.0203	1.931	0.069
Cressbrookdale	-0.0137	0.0096	-1.431	0.170
Great Asby	-0.0076	0.0177	-0.426	0.675
Hay Dale	0.0116	0.0092	1.258	0.224
Rodborough	-0.0707	0.0341	-2.074	0.053
Smardale	0.0333	0.0299	1.114	0.280
Thrislington	-0.0229	0.0223	-1.025	0.319
***model*:** quadrat species richness ~ maximum soil depth				
site	**estimate**	**SE**	**t-value**	**p**
Arnside	0.0059	0.0254	0.230	0.820
Barnack	-0.0036	0.0203	-0.176	0.862
Brockadale	-0.0154	0.0142	-1.085	0.292
Cleeve Common	-0.0205	0.0179	-1.147	0.267
Crook Peak	0.0055	0.0078	0.706	0.489
Cressbrookdale	-0.0169	0.0087	-1.936	0.069

Table 4.9 continued over...

... Table 4.9 continued

model*: quadrat species richness ~ maximum soil depth *cont.				
site	**estimate**	**SE**	**t-value**	**p**
Great Asby	0.0088	0.0141	0.626	0.539
Hay Dale	0.0053	0.0072	0.732	0.474
Rodborough	-0.0454	0.0194	-2.345	0.031
Smardale	0.0065	0.0154	0.420	0.680
Thrislington	-0.0331	0.0336	-0.984	0.338
***model*: quadrat species richness ~ mean quadrat soil depth**				
site	**estimate**	**SE**	**t-value**	**p**
Arnside	0.0185	0.0304	0.608	0.551
Barnack	-0.0157	0.0205	-0.767	0.453
Brockadale	-0.0257	0.0180	-1.429	0.170
Cleeve Common	-0.0137	0.0209	-0.654	0.522
Crook Peak	0.0387	0.0181	2.140	0.046
Cressbrookdale	-0.0164	0.0100	-1.633	0.120
Great Asby	0.0028	0.0154	0.184	0.856
Hay Dale	0.0111	0.0085	1.312	0.206
Rodborough	-0.0521	0.0244	-2.140	0.046
Smardale	0.0153	0.0239	0.640	0.530
Thrislington	-0.0533	0.0388	-1.375	0.186
***model*: quadrat species richness ~ soil depth range**				
site	**estimate**	**SE**	**t-value**	**p**
Arnside	-0.0243	0.0265	-0.918	0.371
Barnack	0.0287	0.0321	0.894	0.383
Brockadale	-0.0021	0.0158	-0.131	0.897
Cleeve Common	-0.0819	0.0340	-2.407	0.027
Crook Peak	0.0003	0.0082	0.037	0.971
Cressbrookdale	-0.0124	0.0154	-0.829	0.418
Great Asby	0.0402	0.0221	1.819	0.086
Hay Dale	-0.0027	0.0098	-0.273	0.788
Rodborough	-0.0525	0.0316	-1.659	0.114
Smardale	-0.0022	0.0182	-0.121	0.905
Thrislington	0.0060	0.0200	0.302	0.766

The five sites where quadrat species richness increased with mean soil depth included the three lowest mean site soil depth values (Arnside 4.25 cm, Smardale 4.72 cm, Crook Peak 6.08 cm), Great Asby mean site soil depth (8.15 cm) below overall mean (8.33 cm), and Hay Dale (mean site soil depth 9.82 cm). Four of these five sites had a large proportion of quadrats with mean depth of less than 8 cm (Arnside 100%; Smardale 96%; Crook Peak 75%; Great Asby 50%), and a number of quadrats with very shallow mean soil depths of less than 3 cm (Arnside 10%; Crook Peak 20%; Smardale 25% (of which 3 were less than 2 cm)); Hay Dale was anomalous with only 9 of the 20 quadrats (45%) having mean soil depth of less than 8 cm, and none less than 5 cm. This characteristic was not restricted to these five sites, as Barnack and Rodborough also had 50% of quadrats with mean soil depth of less than 8 cm, indicating that there were other factors beyond soil depth influencing the species richness response.

4.3.3 Influence of soil depth metrics on species diversity

Associated hypothesis:

H4.2 Plant communities will be more diverse on shallower soils

The relative influence of environmental variables on site species diversity was investigated using the calculated values of Simpson's Index of Diversity, as described in section 4.3.1.4. Generalised linear models indicated that site species diversity increased with increasing minimum soil depth (t = 2.338, 9 df, p = 0.044) (Table 4.10), but was not significantly influenced by the other soil depth metrics.

Table 4.10 Output from generalised linear models of influence of soil depth metrics on Simpson's Index of Diversity. All models had residual variance calculated on 9 df.

***model*:** Simpson's Index of Diversity ~ site-level soil metrics				
metric	**estimate**	**SE**	**t-value**	**p**
minimum	0.1117	0.0478	2.338	0.044
maximum	0.0111	1.0128	0.866	0.409
mean	0.0450	0.0312	1.443	0.183
range	0.0046	0.0119	0.382	0.712
SD	0.0020	0.0788	0.025	0.981

4.3.4 Influence of rainfall, temperature and atmospheric pollutant deposition on species richness and diversity

Of the non-soil-related environmental variables, mean annual temperature and mean annual rainfall accounted for the most variance in site species richness (Table 4.5). Sites with higher mean annual temperatures were more species-rich (Spearman's *rho* = 0.69, r^2 = 0.48, n = 11, p = 0.02); and site species richness declined with increasing mean annual rainfall (Spearman's *rho* = -0.64, r^2 = 0.41, n = 11, p = 0.03). Species richness and Simpson's Index of Diversity were positively correlated with mean total N and NOx deposition; species richness declined with increasing NHy deposition, whereas the diversity index increased with increasing NHy. Site species richness showed no significant correlation with any of the N-related variables, SO_2 or acid deposition.

Generalised linear models supported the correlation findings (Table 4.11). Site species richness declined with increasing precipitation, and increased with increasing temperature (Figure 4.12).

Table 4.11 Output from generalised linear models investigating the influence of rainfall, temperature and nitrogen-deposition on site species richness.

	***model*: site SR ~ environmental variable**				**confidence interval**	
variable	**estimate**	**SE**	**t-value**	**p**	**2.5%**	**97.5%**
annual rainfall	-0.0002	0.0001	-2.002	0.0762	-0.0004	$-7.16e^{-06}$
summer rainfall	-0.0035	0.0020	-1.817	0.0693	-0.0074	0.0002
annual temp	0.0960	0.0528	1.817	0.0692	-0.0071	0.1999
summer temp	0.0650	0.0369	1.763	0.1117	-0.0067	0.1380
N deposition	-0.0014	0.0092	-0.157	0.879	-0.0198	0.0162
Nox	$-3.00e^{-05}$	0.0142	-0.002	0.998	-0.0280	0.0278
NHy	-0.0122	0.0764	-0.159	0.877	-0.1605	0.1391
Nox:NHy ratio	0.0153	0.0362	0.421	0.683	-0.0567	0.0853

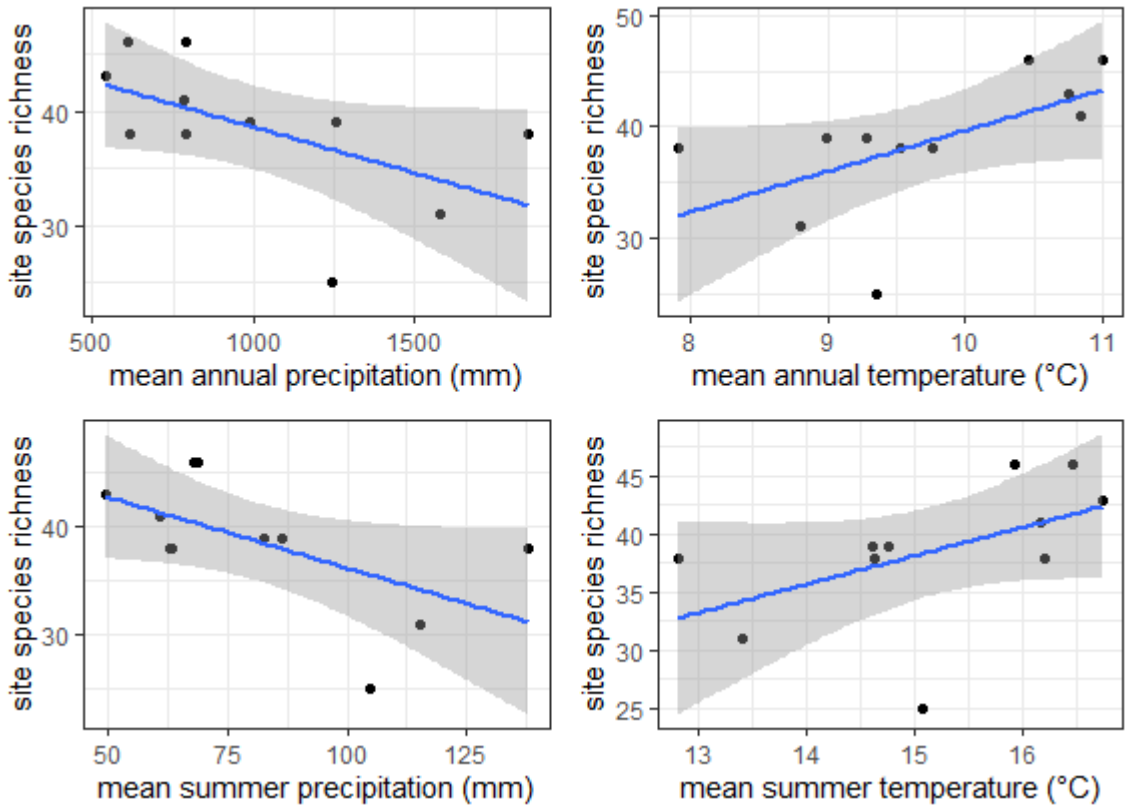


Figure 4.12 Regression of site species richness against mean annual precipitation (R^2 = 0.24, p = 0.07, y = 46.62 - 0.008x), mean summer precipitation (R^2 = 0.26, p = 0.06, y = 49.28 - 0.13x), mean annual temperature (R^2 = 0.26, p = 0.06, y = 3.01 + 3.66x) and mean summer temperature (R^2 = 0.17, p = 0.11, $y = 1.35 + 2.45x$). Shaded areas are 95% confidence intervals.

Simpson's Index of Diversity declined with increasing mean rainfall (mean summer rainfall: t = -2.500, 9 df, p = 0.034; mean annual rainfall: t = -1.799, 9 df, p = 0.109).

***model*: Simpson's Index of Diversity ~ rainfall and temperature variables**				
metric	**estimate**	**SE**	**t-value**	**p**
annual rainfall	-0.0003	0.0002	-1.799	0.106
summer rainfall	-0.0061	0.0358	-0.169	0.866
annual temperature	0.1177	0.0820	1.436	0.185
summer temperature	0.0868	0.0629	1.380	0.201
N deposition	0.0176	0.0151	1.162	0.275
Nox	0.0320	0.0219	1.463	0.178
NHy	0.1828	0.1124	1.625	0.139
Nox:NHy ratio	0.0092	0.0622	0.148	0.886

Total nitrogen deposition was found to exceed the critical load lower limit for all sites barring Smardale; total nitrogen deposition at two sites (Cressbrookdale and Hay Dale) was in excess of

the upper limit of the critical load. None of total nitrogen, NOx or NHy deposition rates had a significant effect on site species richness or Simpson's Index of Diversity.

4.3.5 Influence of soil metrics and environmental variables on plant group richness

Richness within the plant groups was assessed for correlations with environmental variables (Table 4.12, Figure 4.13). There were insufficient data points for woody species to enable further analysis beyond frequency. Between-group correlations indicated that forb and legume richness had a strong positive relationship (Spearman's *rho* = 0.773, p = 0.005, n = 11); increases in graminoid richness were related to declines in legume and forb richness, though these were not significant (graminoid-legume: Spearman's *rho* = -0.334, p = 0.315, n = 11; graminoid-forb: Spearman's *rho* = -0.183, p = 0.590, n = 11).

Table 4.12 Spearman's rank correlations between plant group richness and environmental variables. For all cases, n = 11.

	graminoid richness		**forb richness**		**legume richness**	
variable	***rho***	**p**	***rho***	**p**	***rho***	**p**
latitude	-0.498	0.119	-0.371	0.262	-0.264	0.433
longitude	-0.171	0.616	0.471	0.143	0.301	0.368
elevation	0.401	0.222	-0.618	0.043	-0.871	0.001
slope	0.100	0.769	-0.167	0.624	0.021	0.950
min soil	-0.158	0.644	0.260	0.440	0.068	0.844
max soil	0.381	0.247	0.490	0.126	0.418	0.201
mean soil	0.115	0.736	0.339	0.308	0.212	0.532
soil range	0.494	0.122	0.319	0.339	0.227	0.503
soil SD	0.420	0.198	0.204	0.547	-0.054	0.874
annual rain	0.161	0.636	-0.746	0.008	-0.744	0.009
summer rain	-0.069	0.840	-0.590	0.056	-0.438	0.178
annual temp	0.083	0.808	0.664	0.026	0.692	0.018
summer temp	0.115	0.736	0.517	0.103	0.532	0.092
total N	-0.263	0.435	0.041	0.904	0.443	0.173
NOx	-0.235	0.487	0.150	0.658	0.231	0.495
NHy	-0.124	0.716	-0.128	0.707	0.358	0.280
Nox:NHy ratio	-0.069	0.840	0.266	0.430	-0.052	0.880
acid	-0.152	0.655	-0.238	0.481	0.155	0.648
SO_2	-0.055	0.872	-0.124	0.717	0.066	0.847

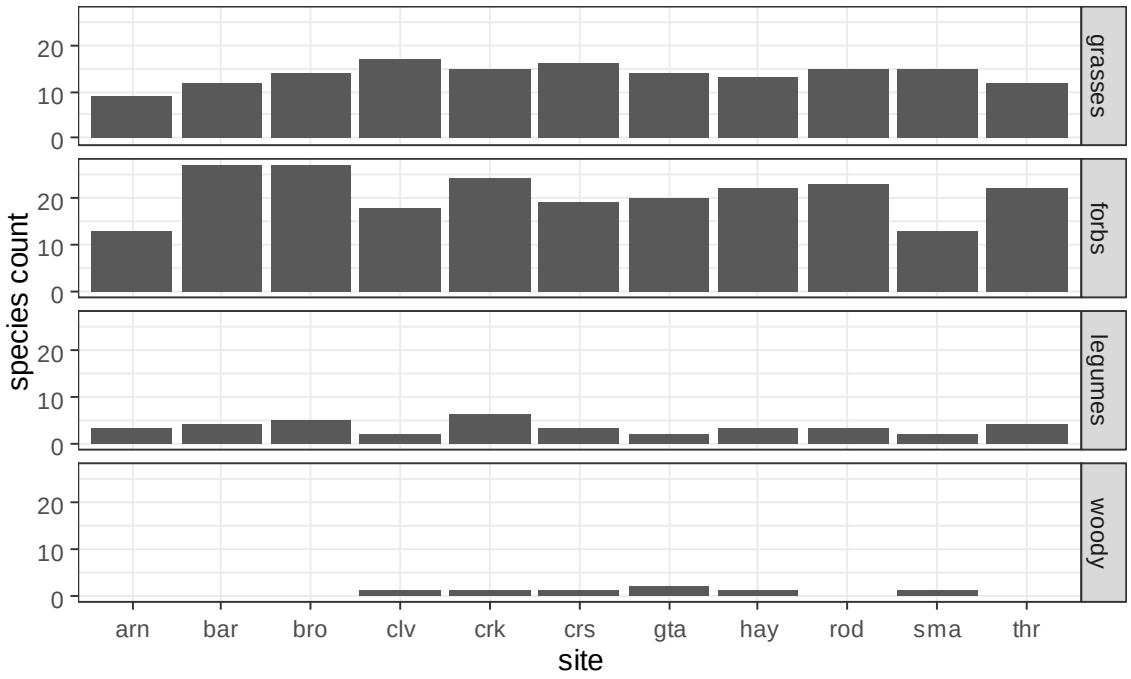


Figure 4.13 Plant group richness by site.

The influence of soil depth metrics, climate and deposition variables on plant group richness were further investigated via generalised linear models (Table 4.13). Richness in all three plant groups was lower on sites with high mean summer rainfall, though this was significant only for forb and legume species counts. Warmer sites were found to support more species from all three plant groups; again, significantly so for forb and legume species, but not for graminoid species.

Generalised linear models indicated that graminoid and legume species counts were negatively associated with increasing minimum soil depth (not significantly); all other relationships with soil depth metrics for the three main plant groups were positive and varied in degree, with forb species richness increasing significantly on sites with higher mean soil depth, and legume species richness being higher on sites with greater maximum soil depth, and with a wider range of soil depth across the site.

Graminoid species were negatively sensitive to all nitrogen deposition variables; forb and legume species were variable in their response. Forb species richness showed a tendency to decline with increased total nitrogen and NHy deposition, and to be favoured by Nox deposition; legume species counts tended to increase under increasing N deposition but to be lower where the Nox:NHy ratio was higher. None of the correlations between plant group richness and atmospheric pollutant deposition were significant.

Table 4.13 Output from generalised linear models for the influence of environmental variables on plant group richness. For all models, n = 11.

***model*:** graminoid species richness ~ environmental variables				
metric	**estimate**	**SE**	**t-value**	**p**
minimum soil depth	-0.0063	0.0349	-0.179	0.862
maximum soil depth	0.0097	0.0071	1.364	0.206
mean soil depth	0.0124	0.0206	0.599	0.564
soil depth range	0.0083	0.0065	1.269	0.236
SD (soil depth)	0.0704	0.0423	1.662	0.131
annual rainfall	$-1.07e^{-05}$	$1.26e^{-04}$	-0.085	0.934
summer rainfall	-0.0007	0.0020	-0.367	0.722
annual temperature	0.0122	0.0551	0.222	0.830
summer temperature	0.0080	0.0423	0.188	0.855
N deposition	-0.0017	0.0093	-0.182	0.860
Nox	-0.0106	0.0141	-0.750	0.473
NHy	-0.0433	0.0761	-0.569	0.583
Nox:NHy ratio	-0.0077	0.0372	-0.208	0.840
***model*:** forb species richness ~ environmental variables				
metric	**estimate**	**SE**	**t-value**	**p**
minimum soil depth	0.0452	0.0475	0.953	0.365
maximum soil depth	0.0161	0.0096	1.670	0.129
mean soil depth	0.0570	0.0247	2.311	0.046
soil depth range	0.0116	0.0920	1.251	0.243
SD (soil depth)	0.0524	0.0662	0.792	0.449
annual rainfall	-0.0004	0.0001	-2.443	0.037
summer rainfall	-0.0058	0.0024	-2.421	0.039
annual temperature	0.1471	0.0644	2.284	0.048
summer temperature	0.1034	0.0525	1.968	0.081
N deposition	-0.0022	0.0132	-0.164	0.873
Nox	0.0118	0.0202	0.583	0.574
NHy	-0.0031	0.1105	-0.028	0.978
Nox:NHy ratio	0.0490	0.0491	0.998	0.345

Table 4.13 continued over ...

...Table 4.13 continued

***model*:** legume species richness ~ environmental variables				
metric	**estimate**	**SE**	**t-value**	**p**
minimum soil depth	-0.0115	0.0829	-0.139	0.893
maximum soil depth	0.0325	0.0126	2.581	0.030
mean soil depth	0.0355	0.0492	0.717	0.491
soil depth range	0.0274	0.0121	2.277	0.049
SD (soil depth)	0.0368	0.1102	0.334	0.746
annual rainfall	-0.0006	0.0002	-2.595	0.029
summer rainfall	-0.0084	0.0042	-1.979	0.079
annual temperature	0.2888	0.0884	3.268	0.010
summer temperature	0.1865	0.0825	2.261	0.050
N deposition	0.0026	0.0217	0.118	0.908
Nox	0.0085	0.0342	0.250	0.808
NHy	0.1517	0.1850	0.820	0.433
Nox:NHy ratio	-0.0237	0.0884	-0.268	0.795

Grass:herb ratio was calculated for each site as the proportionate frequency of graminoid species to non-graminoids in the sward (i.e. grass/(forb + legume). This ratio is considered a useful indicator of high levels of grasses with a concomitant loss of broad-leaved herbaceous species (Robertson and Jefferson, 2000), due to the rapid response of competitive grass species to increased nitrogen supply, and has been seen not only to increase in the face of increased nutrient supply but also to decrease if that supply is subsequently withdrawn (Bobbink and Willems, 1993; Willems and Nieuwstadt, 1996).

It was hoped that the grass:herb ratio might be more sensitive to community responses to the nitrogen deposition variables: this proved not to be the case (Table 4.14). There was, however, an indication that higher maximum soil depths on site had a significant negative impact on the grass:herb ratio, i.e. higher maximum soil depths favoured forb and legume species over grass species abundances. Grass:herb ratio showed a negative trend with all soil depth metrics; also with temperature and all nitrogen-related variables, though none of these were found to be significant at $p<0.05$ level

Table 4.14 Output from generalised linear models of grass:herb ratio response to environmental variables (as listed). For all models, null deviance was calculated on 10 degrees of freedom; residual deviance on 9 degrees of freedom.

***model*:** grass:herb ratio ~ environmental variables				
metric	**estimate**	**SE**	**t-value**	**p**
minimum soil depth	-0.0439	0.0562	-0.780	0.455
maximum soil depth	-0.0252	0.0110	-2.298	0.047
mean soil depth	-0.0337	0.0314	-1.076	0.310
soil depth range	-0.0187	0.0108	-1.728	0.118
SD (soil depth)	-0.0555	0.0752	0.738	0.479
annual rainfall	0.0003	0.0002	1.587	0.147
summer rainfall	0.0051	0.0026	1.940	0.084
annual temperature	-0.1561	0.0732	-2.134	0.062
summer temperature	-0.0946	0.0611	-1.549	0.156
N deposition	-0.0088	0.0153	-0.572	0.581
Nox	-0.0096	0.0234	-0.412	0.690
NHy	-0.0792	0.1232	-0.642	0.537
Nox:NHy ratio	-0.0024	0.0609	-0.040	0.969

4.3.6 Species richness response to the interaction of soil depth with other environmental variables

Associated hypothesis:

H4.6 Soil depth will act to modify the community species richness response to other environmental variables

In order to investigate the interaction effect of soil depth metrics on community response to other environmental variables (with reference to species richness), generalised linear models that included an interaction term were employed. All models outputs and interaction plots can be found in Appendix 4.

The general model structure was:

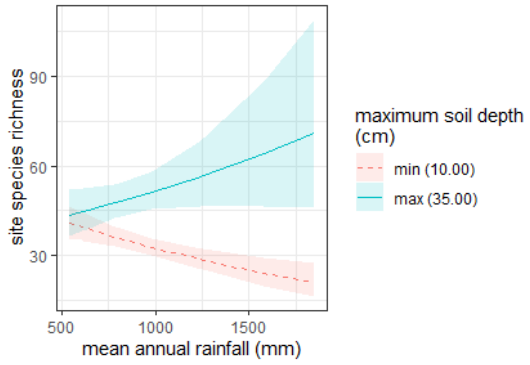
*model: species richness ~ soil depth metric * other environmental variable*

Plots of the marginal effects of the interaction terms of these models indicated that the species richness response to rainfall, temperature and nitrogen deposition were dependent on the level of the soil metrics investigated.

Site species richness responses to mean annual and summer rainfall, and mean annual temperature were found to change depending on the level of maximum soil depth, soil depth range and soil depth SD (Table 4.15).

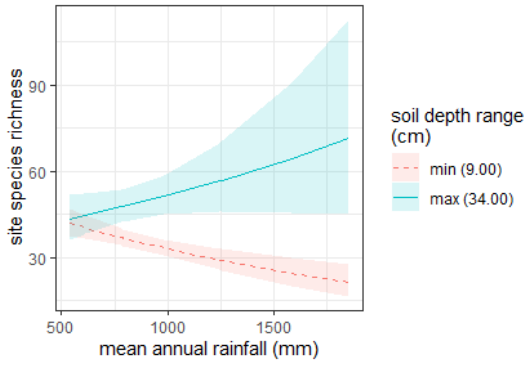
Table 4.15 Site species richness response to mean annual rainfall, mean annual temperature and mean summer rainfall as modified by soil depth metrics where generalised linear models returned a significant (at p<0.05) interaction term. Plots are of marginal effects of interaction term in generalised linear models with format as stated above. For all models, null deviance was calculated on 10 df; residual deviance calculated on 7 df. Metrics have been abbreviated as follows: ann R, annR = annual rainfall; ann T, annT = annual temperature, sum R, sumR = summer rainfall, max = maximum soil depth, SD = standard deviation of soil depth measurements. Interaction between factors is denoted by a colon :.

SR ~ annual rainfall * maximum soil depth



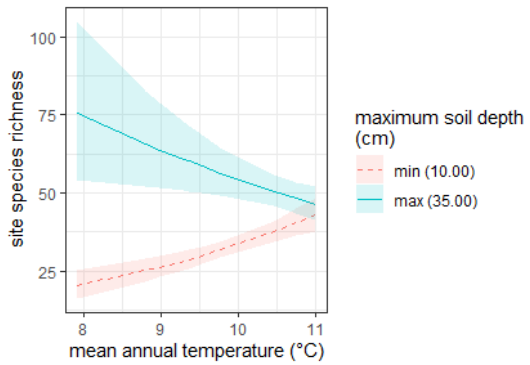
metric	est	SE	t-value	p
ann R	$8.6e^{-04}$	$2.6e^{-04}$	-3.277	0.014
max	$1.6e^{-02}$	$1.2e^{-02}$	-1.352	0.219
annR:max	$3.5e^{-05}$	$1.3e^{-05}$	2.651	0.033

SR ~ annual rainfall * site soil range



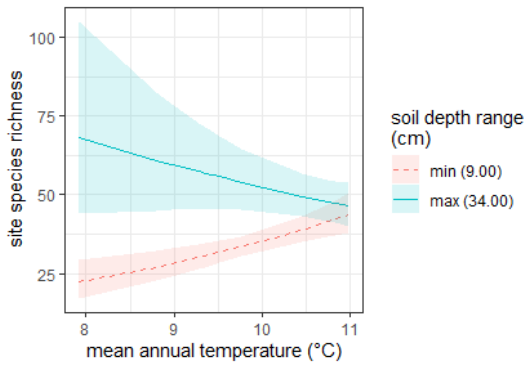
metric	est	SE	t-value	p
ann R	$-8.4e^{-04}$	$2.4e^{-04}$	-3.462	0.011
range	$-1.8e^{-02}$	$1.2e^{-02}$	-1.549	0.165
annR:range	$3.6e^{-05}$	$1.3e^{-05}$	2.706	0.030

SR ~ annual temperature * maximum soil depth



metric	est	SE	t-value	p
ann R	0.406	0.090	4.519	0.003
max	0.180	0.043	4.192	0.004
annT:max	-0.016	0.004	-3.900	0.006

SR ~ annual temperature * site soil range

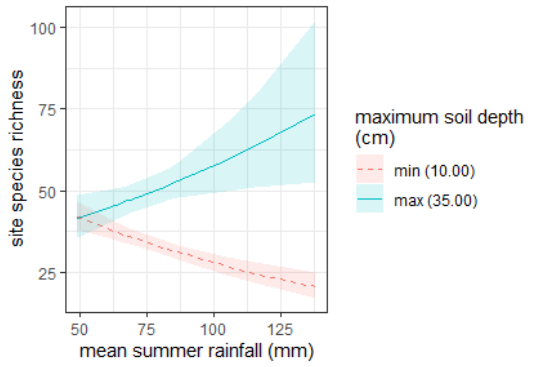


metric	est	SE	t-value	p
ann R	0.344	0.104	3.298	0.013
range	0.154	0.054	2.860	0.024
annT:range	-0.014	0.005	-2.676	0.032

Table 4.15 continued over ...

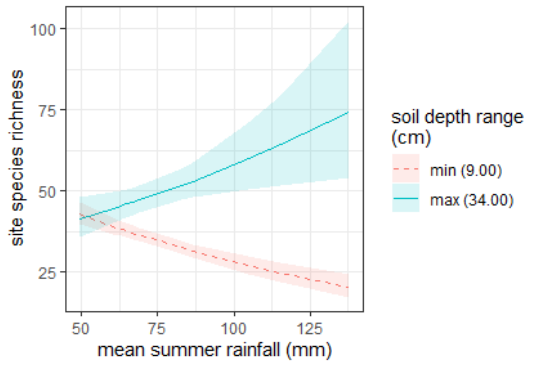
...Table 4.15 continued

SR ~ summer rainfall * maximum soil depth



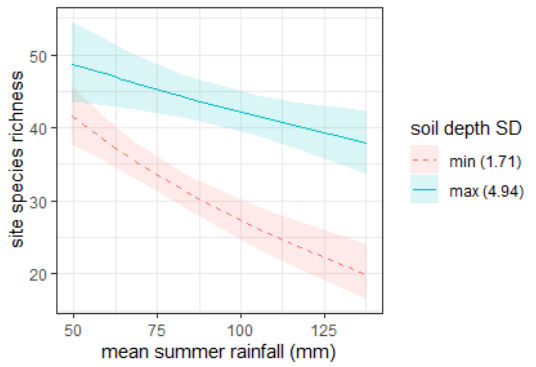
metric	est	SE	t-value	p
sum R	-0.014	0.003	-4.728	0.002
max	-0.029	0.012	-2.445	0.044
sumR:max	$6e^{-04}$	$2e^{-04}$	3.782	0.007

SR ~ summer rain * site soil range



metric	est	SE	t-value	p
sum R	-0.014	0.003	-5.476	0.001
range	-0.031	0.011	-2.931	0.022
sumR:range	$6e^{-04}$	$1e^{-04}$	4.233	0.004

SR ~ summer rainfall * soil depth SD



metric	est	SE	t-value	p
sum R	-0.011	0.002	-4.772	0.002
max	-0.033	0.054	-0.615	0.558
sumR:max	$1.7e^{-03}$	$6e^{-04}$	2.778	0.027

It has already been seen that maximum soil depth and range of soil depth had significant influence on site species richness (section 4.3.2.1), and this influence was also found in interactions with rainfall and temperature variables. Site species richness was higher with increasing rainfall on sites with a wider range of soil depths; conversely, site species richness declined with increasing rainfall on sites with narrower ranges of soil depth. Site species richness responses to changes in mean annual temperature also depended on the range of soil depths on a site, where sites with wider ranges of soil depth had lower species richness as temperature increased. Soil depth range was closely correlated with maximum soil depth,

which had the same pattern of influence on site species richness in its interaction with rainfall and temperature variables.

Mean soil depth was neither a significant factor in site species richness models, nor did it take part in significant interactions with rainfall and temperature variables; none of the models that included minimum site soil depth had significant interaction terms. Plots of interaction term marginal effects for models including mean and minimum soil depths, however, showed crossover or converging interactions for all models barring the interaction of minimum soil depth and Nox/NHy ratio.

The results from these models indicate some dependency of the species richness response on soil depth metrics. The interaction plots indicate that species richness was generally greater on sites with more variable soil depth, as expressed by soil depth range. These results suggest that soil depth may have value as an explanatory variable to explain species richness. Mean soil depth appears to modify the effect of temperature, which will be an important factor as global temperatures increase under climate change, and variation in soil depth will also be an important factor in maintaining diversity under predicted changes in rainfall patterns.

4.3.7 Community attributes

4.3.7.1 Life history

The majority of species were perennials (88 of 111), fifteen were annuals and seven species (all forbs) were biennials. Brockadale and Thrislington had the most biennial species (four species at both sites). These are species that occur as early successional species generally in open vegetation that is intermittently disturbed (De Jong, Klinkhamer and Metz, 1987). Both Brockadale and Thrislington experience moderate to localised heavy visitor pressure; both sites are also managed by grazing, which has been associated with greater habitat diversity, particularly in the provision of heterogenous microsites caused by trampling by stock animals, allowing for higher recruitment from seed set. Breakdown by life history of number of species in each plant group is presented in Table 4.16; breakdown by site is given in Table 4.17.

Table 4.16 Number of species according to life history (total n = 111).

plant group	perennial	annual	biennial
grasses	25	3	0
forbs	53	9	7
legumes	8	3	0
woody	2	0	0

Quadrats at Arnside Knott and Great Asby Scar contained only perennial species when surveyed, i.e. no annuals or biennials. Crook Peak had the highest number of annuals (13 out of 46 species), split between grasses (two species), forbs (eight species) and legume (three species) plant groups; all other sites had between no and three annual species.

Table 4.17 Number of species by plant group and life history. Site abbreviations are as given in Table 4.1. Number of species per life history class is given by nP (perennial), nA (annual), nB (biennial). Within each plant group, the number of species in each life history category is given by P (perennial), A (annual), B (biennial).

site	nP	nA	nB	site SR	grasses		forbs			legumes		woody
					P	A	P	A	B	P	A	P
arn	25	0	0	25	9	0	13	0	0	3	0	0
bar	38	3	2	43	12	0	23	2	2	3	1	0
bro	40	2	4	46	14	0	22	1	4	4	1	0
clv	36	1	1	38	16	1	17	0	1	2	0	1
crs	38	0	1	39	16	0	18	0	1	3	0	1
crk	32	13	1	46	13	2	15	8	1	3	3	1
gta	38	0	0	38	14	0	20	0	0	2	0	2
hay	36	1	2	39	13	0	19	1	2	3	0	1
rod	37	3	1	41	14	1	20	2	1	3	0	0
sma	29	1	1	31	14	1	12	0	1	2	0	1
thr	31	3	4	38	12	0	16	2	4	3	1	0

4.3.7.2 Grime C-S-R signatures

Associated hypotheses:

H4.4 Plant communities on shallower soils will have a higher stress-tolerant element in the C-S-R signature

H4.5 Plant communities will have a higher competitive element in the C-S-R signature in association with a) higher rainfall, b) higher deposition rates of atmospheric nitrogen, or c) deeper soil

Frequency-weighted C-S-R signatures were calculated for each site (Table 4.18) in order to investigate possible functional shifts in the grassland communities in association with environmental variables (see tool developed by *Hunt* et al. *(2004)*). Crook Peak had a slightly different C-S-R signature to the other ten sites, due to the high proportion of annuals (28%), all of which were located in the ruderal region of Grime's C-S-R triangle (R/CR, R/CSR or R/SR).

Table 4.18 Site functional signatures and scores for individual components of site C-S-R signatures. Grime C = proportion of score that is derived from competitor characteristics of the flora; Grime S = stress-tolerant component; Grime R = ruderal component.

site	functional signature	Grime C	Grime S	Grime R
Arnside	S/CSR	0.235	0.540	0.224
Barnack	S/CSR	0.238	0.582	0.180
Brockadale	S/CSR	0.214	0.571	0.215
Cleeve	S/CSR	0.201	0.596	0.203
Cressbrook	S/CSR	0.190	0.612	0.198
Crook Peak	SR/CSR	0.189	0.457	0.354
Great Asby	S/CSR	0.220	0.588	0.192
Hay Dale	S/CSR	0.184	0.589	0.227
Rodborough	S/CSR	0.225	0.529	0.247
Smardale	S/CSR	0.241	0.602	0.158
Thrislington	S/CSR	0.223	0.559	0.218

Grime R scores had the widest range (0.158 - 0.354, range 0.196); the high score (0.354) was associated with Crook Peak, which had an anomalously high proportion of ruderal species (13 out of 46 species in total). Stress scores consistently made up the highest proportion of the total site C-S-R signatures (0.457 – 0.612), indicating that the communities were composed mainly of species with high stress tolerance; Grime C (competitor) scores were generally low,

with the narrowest range (0.184 – 0.241), suggesting a low proportion of very competitive species. Correlation coefficients for Grime C, S and R components of site C-S-R signatures with soil depth metrics, rainfall, temperature and nitrogen-related variables are given in Table 4.19.

Table 4.19 Spearman's correlation coefficients for proportion of site C-S-R signature relating to competitive (C), stress-tolerant (S) and ruderal (R) species traits. For all correlations, n = 11.

	% Grime C		% Grime S		% Grime R	
variable	**rho**	**p**	**rho**	**p**	**rho**	**p**
min soil	0.0702	0.8376	-0.1854	0.5852	0.2456	0.4667
max soil	-0.7980	0.0039	-0.0734	0.8302	0.3119	0.3504
mean soil	-0.5000	0.1214	0.2455	0.4682	0.0182	0.9676
soil range	-0.6606	0.0269	0.1959	0.5637	0.0091	0.9788
soil SD	-0.5877	0.0573	0.3872	0.2393	-0.2005	0.5545
annual rain	-0.1000	0.7757	0.4455	0.1728	-0.1818	0.5952
summer rain	-0.0636	0.8601	0.3000	0.3711	-0.1818	0.5952
annual temperature	-0.0545	0.8601	-0.6727	0.0281	0.5091	0.1140
summer temperature	-0.0273	0.9460	-0.4364	0.1825	0.2818	0.4021
total N	-0.5364	0.0936	-0.0273	0.9460	0.4182	0.2031
Nox	0.2364	0.4855	-0.3727	0.2606	0.3364	0.3130
NHy	-0.3455	0.2994	-0.1909	0.5763	0.5000	0.1214
Nox/NHy ratio	0.6182	0.0478	-0.1182	0.7343	-0.2273	0.5031

Site Grime C was inversely related to all soil depth metrics barring minimum soil depth, i.e. the site competitor component declined as soil depth became more variable (range and SD) or was generally (mean) or occasionally (maximum) deeper. None of the Grime C, S or R fractions were significantly correlated with mean site soil depth; site Grime C declined as site mean soil depth increased, whereas Grime S and R scores increased.

The competitor fraction (Grime C) of the frequency-weighted site C-S-R signatures were more strongly associated with N-related variables than with climate, and increased with increasing Nox/NHy ratio. Grime C was found to have negative relationships with total N and NHy deposition, but not with NOx. Grime C was significantly associated with NOx:NHy ratio (Spearman's *rho* = 0.6182, p = 0.0478, n = 11), and weakly associated with decreasing total N (Spearman's *rho* = -0.5364, p = 0.0936, n = 11), indicating fewer competitive species on sites receiving higher NHy deposition relative to Nox deposition. In contrast, site Grime S scores (i.e. relating to stress tolerance) were more strongly associated with climate than with nitrogen-related variables, with a significant negative correlation with mean annual temperature (Spearman's *rho* = -0.6727, p = o.0281, n = 11), i.e. sites with lower mean annual temperature

hosted communities that were overall more stress-tolerant. Site ruderal component did not appear to show any pattern to responses, suggesting that ruderal species were influenced by other factors not considered, e.g. disturbance caused by grazing or visitor traffic.

Mean quadrat C-S-R scores were calculated as the mean of each of the competitor, stress-tolerant and ruderal elements of every species present in a 15 cm x 15 cm quadrat. Linear mixed effect models indicated that mean, minimum and maximum soil depth were more important than variation (range, SD) in soil depth for Grime C, S and R trait expression at quadrat level, when unconsidered variation between sites was accounted for (Table 4.20).

Table 4.20 Output from linear mixed effect models of the influence of soil depth metrics on Grime C, S and R components of quadrat-level Grime C-S-R signatures.

***model:* Grime C ~ soil metric + (1|site)**					
soil depth metric	**estimate**	**SE**	**df**	**t-value**	**p**
minimum	$2.583e^{-03}$	$9.921e^{-04}$	216	2.603	0.010
maximum	$1.457e^{-03}$	$7.241e^{-04}$	217	2.013	0.045
mean	$2.454e^{-03}$	$9.619e^{-04}$	214	2.551	0.011
range	$1.172e^{-04}$	$8.958e^{-04}$	217	0.131	0.896
SD	$5.249e^{-04}$	$2.156e^{-03}$	217	0.243	0.808
***model:* Grime S ~ soil metric + (1|site)**					
soil depth metric	**estimate**	**SE**	**df**	**t-value**	**p**
minimum	-0.0044	0.0017	218	-2.582	0.011
maximum	-0.0033	0.0012	218	-2.661	0.008
mean	-0.0052	0.0016	218	-3.143	0.002
range	-0.0014	0.0015	215	-0.925	0.356
SD	-0.0031	0.0037	215	-0.853	0.395
***model:* Grime R ~ soil metric + (1|site)**					
soil depth metric	**estimate**	**SE**	**df**	**t-value**	**p**
minimum	0.1946	0.0171	217	1.434	0.153
maximum	0.0018	0.0009	216	1.980	0.049
mean	0.0027	0.0012	218	2.205	0.029
range	0.0012	0.0011	213	1.126	0.261
SD	0.0025	0.0026	212	0.940	0.348

Quadrat-level fractions of Grime C, Grime S and Grime R were all significantly influenced by maximum and mean quadrat soil depths; quadrat Grime C and Grime S were also significantly

influenced by minimum soil depth. None of quadrat fractions of Grime C, S or R had a strong relationship with range or standard deviation of soil depths measured at quadrat level. Quadrat Grime C increased significantly with increasing minimum, maximum and mean quadrat soil depth; Grime S declined significantly with increasing minimum, maximum and mean soil depths.

The survey data found a mixed response to soil depth metrics in the stress-tolerant fraction of site C-S-R signatures: site Grime S was negatively associated with minimum and maximum soil depths, and positively with mean soil depth, soil depth range and soil depth SD, though none of these associations were significant at $p < 0.05$. The strongest responses were to soil depth SD (Spearman's *rho* = 0.39), mean soil depth (Spearman's rho = 0.25) and soil depth range (Spearman's *rho* = 0.20). This suggests that soil depth heterogeneity, as expressed by soil depth range, mean and SD, may be more important than absolute soil depth, at the site level. At the finer scale of quadrat level, this pattern was inverted to a degree, with quadrat Grime S being significantly influenced by the minimum, maximum and mean soil depth metrics; the relationship with all soil metrics was negative at quadrat level, when variation by site had been accounted for. Overall, the data supported hypothesis H4.4, that shallower soils would be associated with higher stress-tolerance signatures, though this depended on scale, and appeared to be confounded by other factors at site level.

The hypotheses that higher fractions of Grime C would be associated with increased nitrogen deposition, deeper soil and higher rainfall were investigated further via generalised linear models, which are summarised in Table 4.21. These models indicated that site Grime C was significantly lower on sites with higher total nitrogen deposition. The Grime C fraction of site C-S-R signatures was significantly higher on sites with higher Nox/NHy ratio, though not significantly influenced by Nox or NHy deposition *per se*. The relationship between Grime C and Nox and NHy appear to be in different directions, i.e. Grime C had a positive correlation with Nox, and a negative correlation with NHy. Hypothesis H4.5b (that Grime C would increase with increasing nitrogen deposition) was supported with regard to total nitrogen deposition, also to increasing ratio between Nox and NHy (i.e. where Nox increases in relation to NHy, or *vice versa*).

Soil depth was negatively associated with Grime C at site level, and positively with Grime C at quadrat level; the significance of these associations varied by scale, with soil depth heterogeneity appearing to have a stronger influence at site level than absolute soil depth values, while mean, minimum and maximum soil depths were significantly associated with

Grime C at quadrat level. The data did not, therefore, support hypothesis H4.5c (that Grime C would increase with increasing soil depth) at site level.

Grime C was negatively (and not significantly) associated with both mean annual and mean summer rainfall, which therefore did not support part *a* of hypothesis H4.5.

Table 4.21 Output from generalised linear models of the influence of nitrogen, soil depth and rainfall variables on site Grime C. For all models, residual deviance was calculated on 9 df.

***model:* Grime C ~ nitrogen variables**				
N variable	**estimate**	**SE**	**t-value**	**p**
total N	-0.0021	0.0009	-2.278	0.049
Nox	0.0010	0.0018	0.531	0.608
NHy	-0.0127	0.0089	-1.424	0.188
Nox/NHy ratio	0.0087	0.0038	2.297	0.047
***model:* Grime C ~ soil depth metric**				
soil depth metric	**estimate**	**SE**	**t-value**	**p**
minimum	0.0012	0.0045	0.277	0.788
maximum	-0.0021	0.0007	-2.902	0.018
mean	-0.0030	0.0025	-1.223	0.252
range	-0.0018	0.0007	-2.571	0.030
SD	-0.0117	0.0046	-2.550	0.031
***model:* Grime C ~ rainfall metric**				
metric	**estimate**	**SE**	**t-value**	**p**
annual rainfall	$5.27e^{-06}$	$1.60e^{-05}$	0.331	0.718
summer rainfall	$1.29e^{-04}$	$2.49e^{-04}$	0.518	0.617

4.3.7.3 Ellenberg Indicator Values

Frequency-weighted mean Ellenberg values for each site were calculated for nitrogen (N), moisture (F), light (L) and soil pH (R), and are presented in Table 4.22. All sites held species from a broad spread of levels across all EIVs, though not all levels were represented on each site.

Table 4.22 Frequency-weighted mean Ellenberg Indicator Values (EIV). N = EIV nutrients, R = EIV moisture, L = EIV light, R = EIV reactivity/pH.

site	N	F	R	L
Arnside	3.42	5.09	5.89	6.91
Barnack	2.79	4.32	6.59	7.24
Brockadale	2.93	4.43	6.74	7.24
Cleeve	2.91	4.89	5.77	7.13
Crook Peak	3.58	4.47	6.32	7.23
Cressbrookdale	3.02	4.63	6.13	7.16
Great Asby	2.89	5.14	5.69	7.07
Hay Dale	3.01	4.68	6.23	7.25
Rodborough	3.22	4.46	6.59	7.11
Smardale	3.18	4.47	6.48	6.97
Thrislington	2.88	4.60	6.79	7.21

The plant species present on the eleven sites predominantly indicated for low nutrient conditions, as is characteristic of calcareous grasslands (EIV 1 = extremely infertile, EIV 3 = infertile, EIV 5 = intermediate fertility) (Figure 4.14), which was reflected in the community weighted EIV for nutrient. There was a significant trend for sites with higher EIV signatures for Ellenberg F to have lower species richness ($p < 0.01$), and for high species richness to be associated with high EIV signatures for Ellenberg L ($p < 0.01$). Neither Ellenberg N nor Ellenberg R were significantly associated with species richness.

Ellenberg N (nutrient)

Nearly half of the species (48%) were associated with Ellenberg N level 3 or lower, including the forbs *Galium sterneri* (Arnside and Hay Dale), *Hieracium pilosella* and *Linum catharticum* (both present at all sites but Thrislington); the legumes *Anthyllis vulneraria* (Brockadale, Hay Dale, Rodborough, Thrislington) and *Lotus corniculatus* (all sites); and the grasses *Avenula pratensis* (all sites except Arnside, Crook Peak and Great Asby) and *Sesleria caerulea* (Arnside, Great Asby, Smardale and Thrislington).

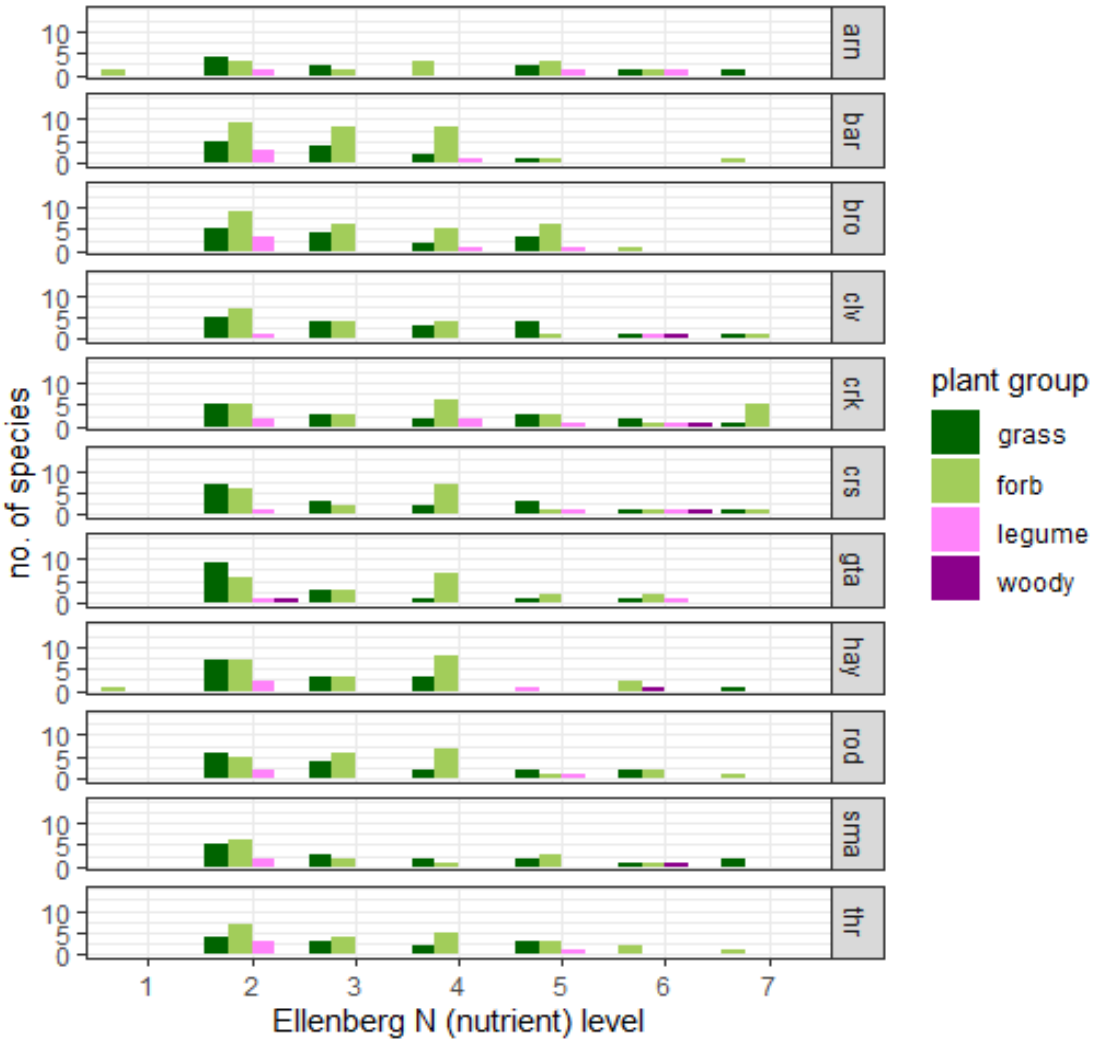


Figure 4.14 Incidence of species within Ellenberg N levels, i.e. numer of species that that Ellenberg N value recorded in survey quadrats on site. Site names are abbreviated as per Table 4.1.

Species indicative of "richly fertile" conditions (Hill *et al.*, 1999) (Ellenberg N level 7), e.g. *Arrhenatherum elatius*, *Bromus sterilis*, *Ranunculus repens* and *Senecio squalidus*, were present on all sites to some degree, barring Brockadale and Great Asby. Crook Peak had the highest EIV for Ellenberg N (EIV of 3.58); this was due to the relatively high number (n = 5) of forb species with EIVs of 7. This site also had the highest number of annual species (13), and was the most southerly of the sites. Ellenberg N was not significantly correlated with any environmental variables, nor with site SR and diversity. There was no significant relationship between Ellenberg N and any of the nitrogen-related variables, so the hypothesised increase in nitrophilous species with increased N deposition was not supported.

Ellenberg F (moisture)

Most species had EIV F (moisture) of 4-5 (Figure 4.15). Moisture conditions on most sites were between 4 and 5, indicating conditions to be dry to damp (EIV 3 = dry site, EIV 5 = fresh soil of average dampness), apart from Great Asby, which had a weighted site EIV of 5.14, and Arnside, with site EIV of 5.09 for moisture. Great Asby was the highest site in terms of altitude, and received the highest mean annual precipitation (2000 mm yr^{-1}). It also offered the greatest surface hetergeneity, with upstanding limestone pavement interspersed with deep sheltered grikes (fissures), and broader open areas of grassland in between. Arnside was not distinguished by any particular environmental variable that might provide a simple explanation for the slightly raised site Ellenberg F score. Community Ellenberg F was strongly associated with both mean annual temperature (negative relationship, $R^2 = 0.42$, $P < 0.05$) and mean annual rainfall (positive relationship, $R^2 = 0.30$, $P < 0.05$).

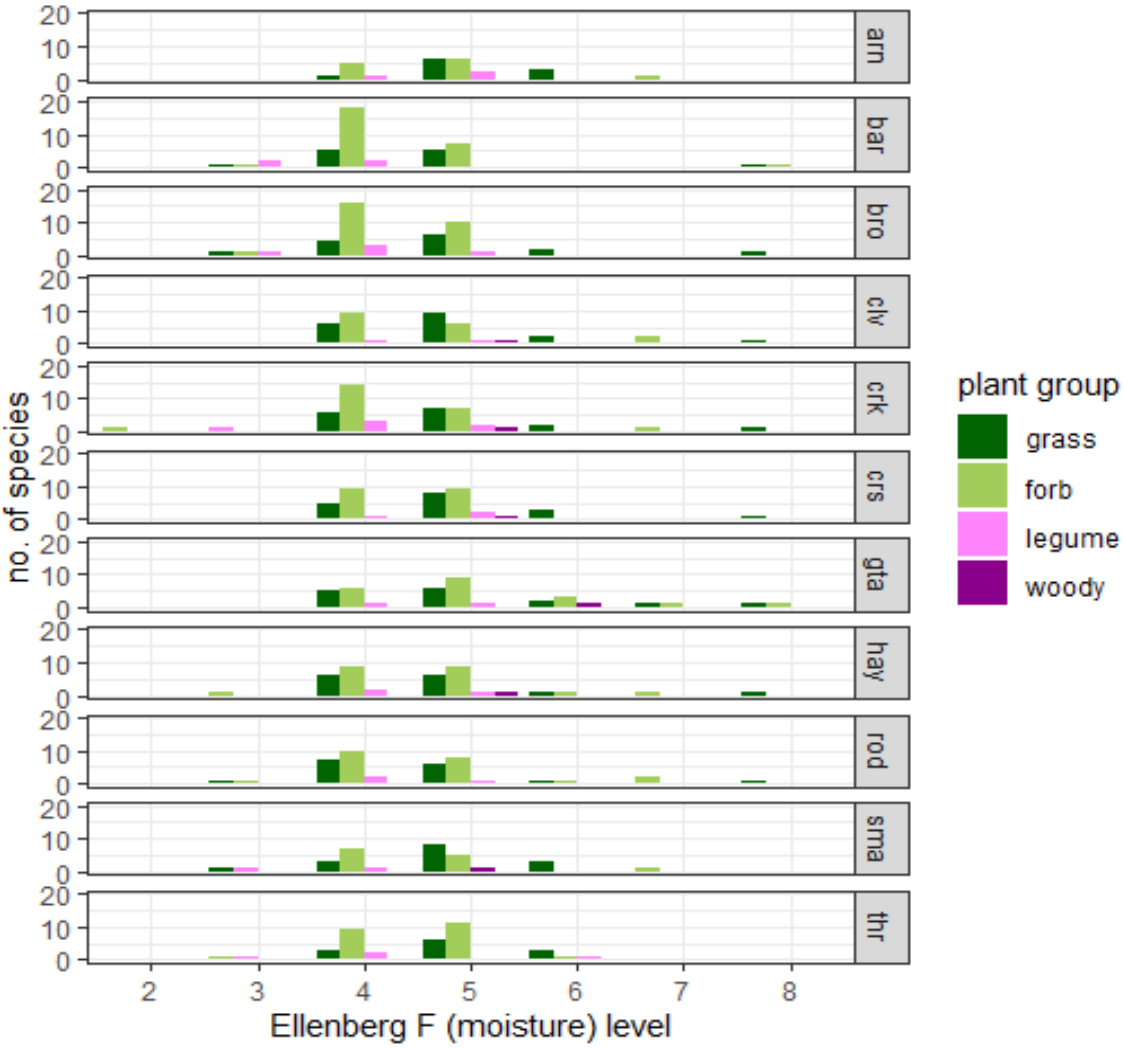


Figure 4.15 Incidence of Ellenberg F levels, i.e. number of species within that EIV level recorded in survey quadrats on site. Site names are abbreviated as per Table 4.1.

Dry-site species with EIV level 3 included legumes *Astragalus danicus* (Barnack only), *Hippocrepis comosa* (Barnack, Brockadale and Smardale), and *Trifolium scabrum* (Thrislington and Crook Peak); also the forbs *Knautia arvensis* (Barnack, Hay Dale and Rodborough) and *Scabiosa columbaria* (Brockadale and Thrislington) and the grass *Brachypodium pinatum* (Barnack, Brockadale, Rodborough and Smardale). *Sedum acre*, a species of rocky outcrops and other similar very dry conditions, was observed at Crook Peak.

A decline in species richness with increased rainfall was echoed in the decline in species richness with an increase in site mean Ellenberg F (moisture) values; this relationship was significant for mean annual ($p < 0.001$) and mean summer ($p < 0.05$) rainfall. Site species richness and diversity were both negatively correlated with Ellenberg F, indicating that drier sites were more diverse and supported a greater number of species. Ellenberg F increased with increasing precipitation (mean annual $p < 0.001$, mean summer $p < 0.05$), and showed a negative relationship with mean temperature (mean annual and mean summer temperatures both $p < 0.05$).

Ellenberg R (pH)

Most species on all sites had an EIV for reaction (i.e. soil pH) of 6 or above (Figure 4.16), which included characteristic species of calcareous grassland such as *Centaurea nigra*, *Polygala vulgaris*, *Cirsium acaule*, *Hieracium pilosella* and habitat specialists *Astragalus danicus* and *Blackstonia perfoliata*.

All sites, however, also supported species of much lower EIVs for R, which indicate less alkaline conditions. All site species lists included species of EIV level 4 (nearly neutral to moderately acid soils (Hill *et al.*, 1999)), all of which were graminoids (*Agrostis capillaris* (5 sites), *Anthoxanthum odoratum* (10 sites), *Carex nigra* (8 sites), *Festuca ovina* (11 sites). Four species of EIV level 3 (species of mainly acid but occasionally nearly neutral soils) were observed (*Luzula multiflora* (Cressbrookdale only), *Nardus stricta* and *Carex pilulifera* (both Great Asby only), and *Potentilla erecta*, which was found on six sites. *Calluna vulgaris* and *Vaccinium myrtillus* are both species of mainly acid soils (EIV level 2), and were observed at Great Asby.

Somewhat surprisingly for sites overlying limestone, all the weighted site Ellenberg values for reaction (i.e. soil pH) indicated mid-range pH between 5 and 7: for Ellenberg R, a value of 5 is an indicator of moderately acid soils, characterised as species "only occasionally found on very acid or on neutral to basic soils", and a value of 7 is "weakly acid to weakly basic" (Hill *et al.*, 1999)).

Part of the definition for calcareous grasslands is that they form predominantly on lime-rich soils with pH between 6.5 and 8.5 (Jefferson, Smith and MacKintosh, 2014), so these slightly low EIVs for reaction/pH may indicate a small shift in plant communities as a result of acidification, though acidic vegetation is common on calcareous substrates in northern UK, where high rainfall leads to organic acidic soils over basic rocks.

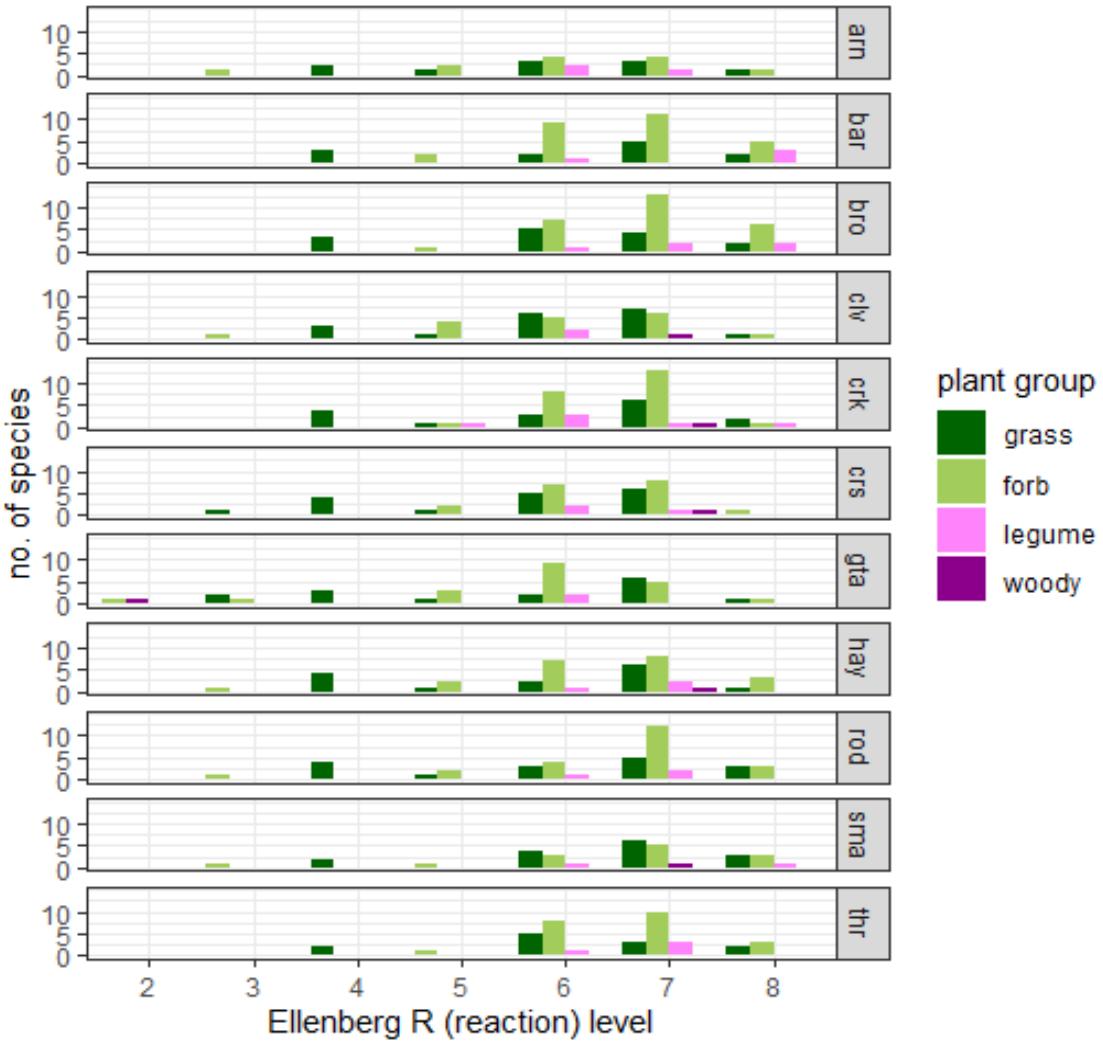


Figure 4.16 Incidence of Ellenberg R levels, i.e. number of species within that EIV level recorded in survey quadrats on site. Site names are abbreviated as per Table 4.1.

Site mean Ellenberg R scores were not significantly correlated with site SR or diversity, nor with any of the nitrogen, acid or SO_2 deposition rates. An inverse relationship with nitrogen deposition might have indicated that plant communities were experiencing an increase in soil acidity at those locations, but this was not borne out by the data. There was a significant decline in site Ellenberg R with increasing annual precipitation ($p < 0.05$); the wetter sites, therefore, being more acid.

Ellenberg L (light)

Most species' EIVs for light were at levels 7 and 8 (Figure 4.17) – these are plants of generally well-lit locations, though level 7 allows for partial shade. Species richness and Simpson's Index of Diversity increased with increasing Ellenberg L (both $p < 0.01$).

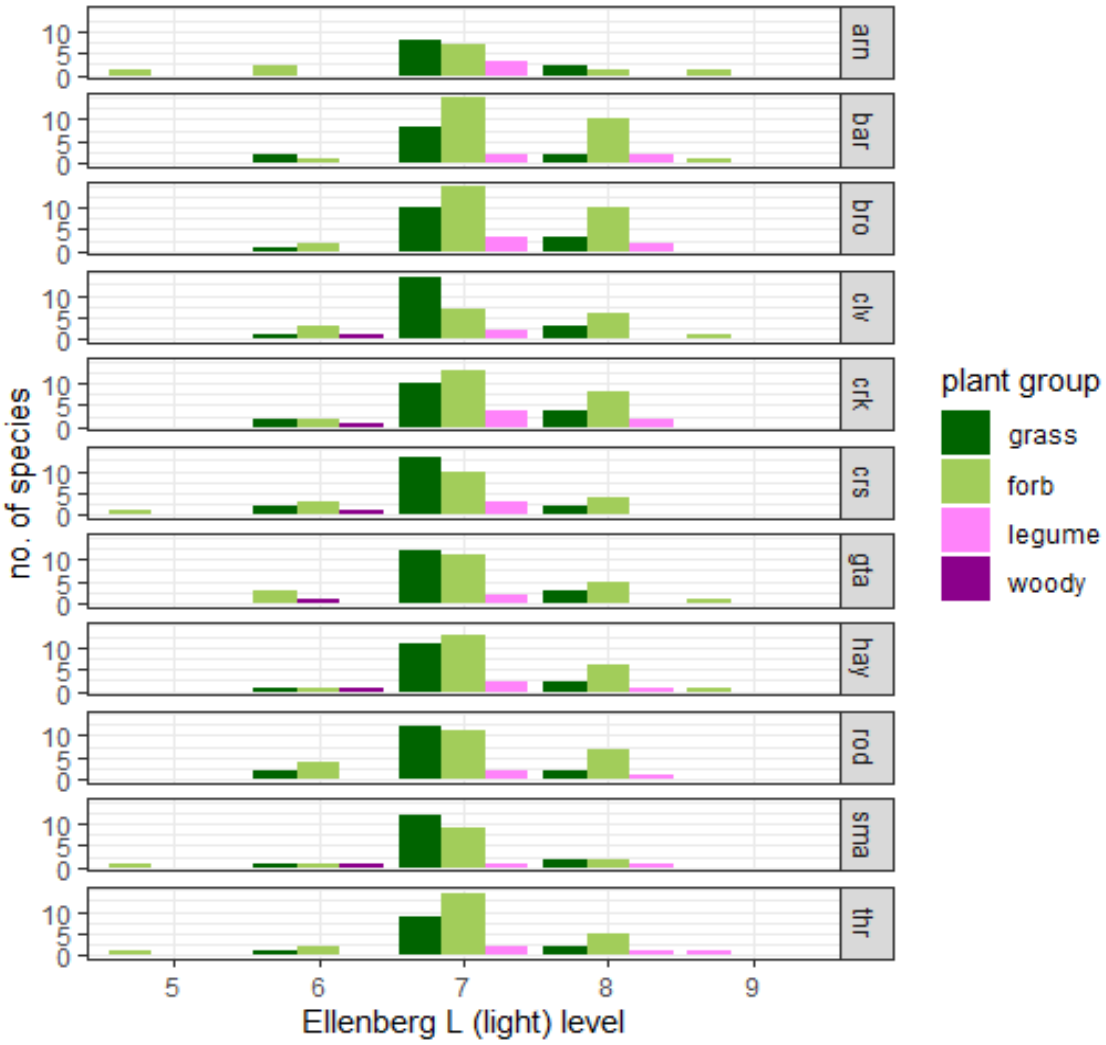


Figure 4.17 Incidence of Ellenberg L levels. Site names are abbreviated as per Table 4.1.

Where Ellenberg L declines with increasing nitrogen, or with increasing Ellenberg N scores, it can indicate an increase in taller species under increased nitrogen deposition, which are shading out low- growing species more characteristic of short-sward grasslands. Again, there was no evidence of this from the data from the eleven surveyed sites, and Ellenberg L was not significantly correlated with any of the nitrogen variables. There was a weak linear relationship between Ellenberg L and mean annual precipitation ($p < 0.05$), but none with mean summer climate metrics, not with mean annual temperature.

4.3.7.4 NMDS ordination

Non-metric multidimensional ordination output on two dimensions achieved convergence with a stress level of 0.1385. Resulting R^2 values were non-metric fit $R^2 = 0.98$, linear fit $R^2 = 0.88$. The primary axis (NMDS1) is the axis of greatest variance, i.e. factors closely associated with this axis explain higher amount of variance than factors less closely associated. Closeness of association can be identified qualitatively; the more parallel a factor vector is to an axis, the more closely associated they are.

Figure 4.18 and Figure 4.19 offer two views of species' distribution in the above NMDS ordination; all species were included in the ordination, but are highlighted in the two figures according to abundance. Figure 4.18 highlights species that were present in 5% or more of the survey quadrats (n = 51). Figure 4.19 highlights the rarer species (n = 60), i.e. those observed in fewer than 5% of survey quadrats. Both figures show environmental factors as blue vectors; species names have been abbreviated – the abbreviations used in the figures are presented in Appendix 3. Where species are overlaid in the plot, they have been listed in inserts and their ordination location indicated by an arrow.

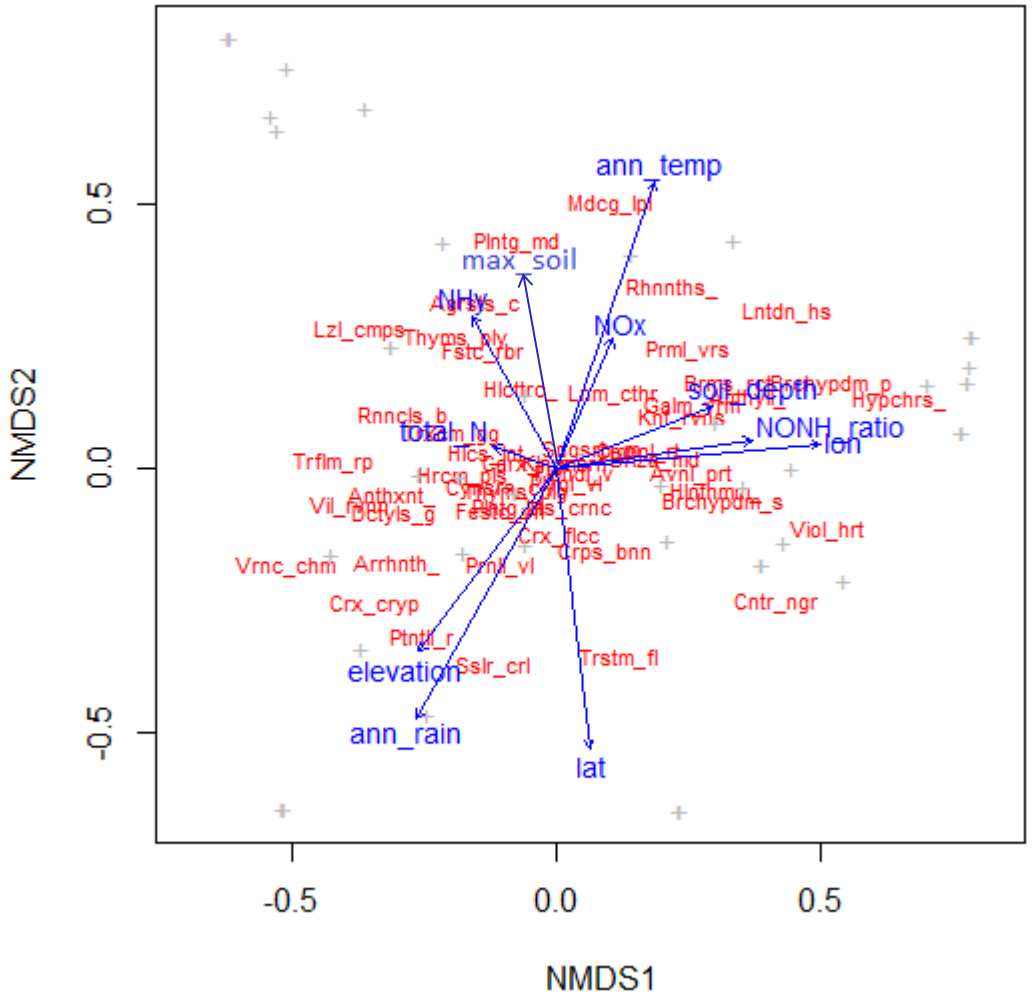


Figure 4.18 NMDS ordination plot for species present on the surveyed calcareous grassland sites. The most abundant species (i.e. species in >5% pf quadrats) are labelled in red; ordination locations for the rare species (i.e. species in <5% of quadrats) are indicated by a grey cross +. Vectors for environmental factors are indicated by blue arrows; their length indicates the relative strength of influence on the ordination. Species names are abbreviated as per Appendix 3.

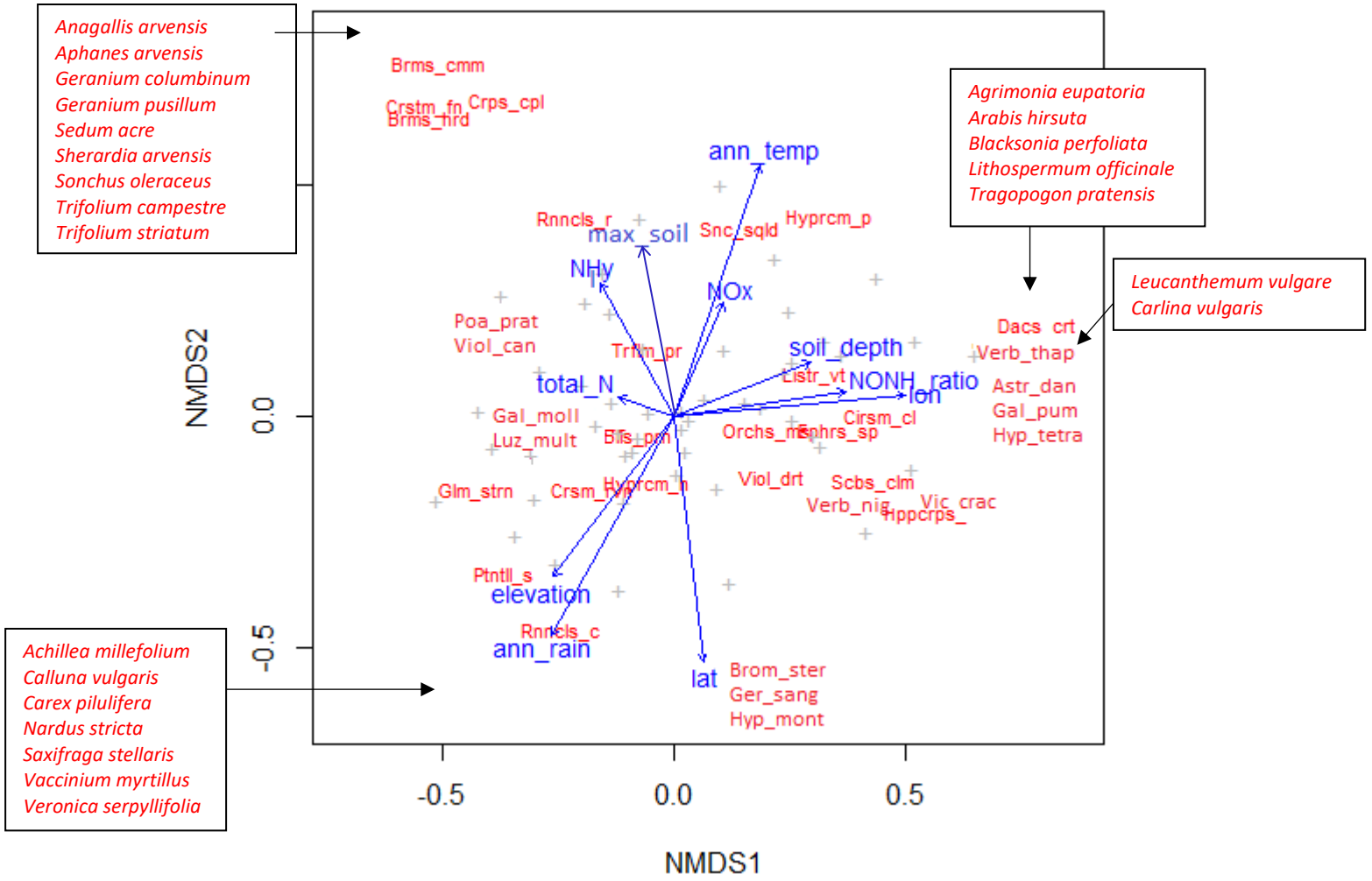


Figure 4.19 NMDS ordination plot for species present on the surveyed calcareous grassland sites. Species present in fewer than 5% of quadrats are labelled in red; more abundant species' ordination locations are indicated by grey crosses +. Species names are abbreviated as per Appendix 3.

Permutation-based p-values indicate the strength of relationship between ordinations and environmental variables (Table 4.23). Mean and minimum soil depth measures were most strongly associated with the primary axis; maximum soil depth and depth range were more strongly associated with the secondary axis; none of these relationships were significant.

Table 4.23 Relationship between NMDS ordination vectors, environmental variables and community characteristics. Output from permutation tests (n = 999) carried out by envfit() function in vegan package in R (Oksanen, 2018), to test goodness of fit by comparing observed value of R^2 to large number of R^2 values derived from repeatedly shuffling the site environmental data. rNMDS1 and rNMDS2 are results of Spearman's rank correlation between NMDS axes and the environmental variables. Ellenberg values are frequency-weighted for all species on site. Significance levels as per Table 3.3.

variable	R^2	p-value	r NMDS1	r NMDS2
latitude	0.77	<0.01 **	0.24	-0.85 **
longitude	0.68	0.01 *	0.84 **	0.14
mean soil depth	0.27	0.29	0.45	0.19
minimum soil depth	0.18	0.49	0.38	0.20
maximum soil depth	0.35	0.17	-0.15	0.51
soil depth range	0.29	0.24	-0.15	0.35
elevation	0.51	0.07	-0.37	-0.46
total N	0.04	0.82	-0.15	0.11
NOx	0.19	0.41	0.19	0.30
NHy	0.29	0.25	-0.25	0.14
NOx:NHy ratio	0.38	0.15	0.66 *	0.19
mean annual temperature	0.89	<0.001 ***	0.11	0.89 ***
mean annual precipitation	0.80	<0.01 **	-0.56	-0.66 *
mean summer temperature	0.83	0.001 ***	0.03	0.81 **
mean summer precipitation	0.82	0.001 ***	-0.45	-0.70 *
Ellenberg N	0.44	0.10	-0.54	0.21
Ellenberg R	0.74	<0.01 **	0.79 **	0.29
Ellenberg F	0.84	<0.01 **	-0.63 *	-0.67 *
Ellenberg L	0.49	0.08	0.41	0.49

Both latitude and longitude had a significant influence on community structure, as did the temperature and rainfall variables (Table 4.23). Correlation tests between NMDS ordination values and environmental variables highlighted the relative influence of each variable on the two axes: from the above plots and the results of correlation tests, it can be seen that the primary axis (NMDS1) was most strongly associated with the NOx:NHy ratio and longitude, and the secondary axis (NMDS2) with latitude, mean temperatures and rainfall. Mean annual

temperature had a stronger influence on community structure ($p < 0.001$) than mean summer temperature ($p < 0.01$).

With regard to functional qualities of the communities, site Ellenberg R was significantly associated with community composition on axis 1 (NMDS1). This may be a reflection of soil pH, but it should be remembered that Ellenberg R is also associated with other traits beyond acidity, including thermal germination requirements (Bartelheimer and Poschlod, 2016). There was no indication from the ordination that nitrogen tolerance/preference or light levels were significantly associated with community composition.

4.4 Discussion

Soil depth and species richness

There was some evidence to support the hypothesis that species richness would increase with increasing soil depth, though this was not a straightforward relationship. Although species richness had no significant relationship with mean soil depth, there were significantly more species on sites with higher maximum soil depths, which intimated that species may be closely associated with quite strictly compartmentalised depths. This echoes the findings of Fridley *et al.* (2011a) at the Buxton climate change experiment, where half of the 25 most common species exhibited affinities with certain soil depths. At the coarse (site) scale, mean soil depth does not give an indication of the degree of heterogeneity in soil depth, and so may not be the best metric for assessing drivers of diversity on these grasslands; range of soil depths (i.e. maximum -minimum) was weakly associated with species richness where mean and minimum soil depth were not.

Analysis of site depth variability, as described by soil depth standard deviation, quantified the interactive relationship between species richness, soil depth and other environmental variables. My results showed that soil depth heterogeneity had a significant additive effect on species richness response to climate variables; greater soil heterogeneity increased species richness, which supports the findings of Fridley *et al.* (2011). Soil depth variation appeared to enhance the rate of change in species richness as a result of response to the interacting variable; drivers that negatively influenced species richness, e.g. precipitation, saw a steeper response curve from species richness when soil depth variability was greater.

At the finer scale, the number of species present in a quadrat was significantly increased where the mean quadrat soil depth was greater; minimum and maximum soil depths recorded for each quadrat were also found to be strongly associated with this localised species richness, with an apparent optimum of minimum depth between 6-8 cm, and maximum depth of between 8 and 10 cm. Up to a depth of 6-8 cm, increasing soil depth appeared to support more species; it may be that increasing soil depth provides greater niche space up to this point, due to increased water and nutrient resources , allowing for mixtures of species with different rooting depths to inhabit that space (Berendse, 1982). The species richness-soil depth associations could be explained in terms of Grime's hump-backed model of species richness along a resource gradient (Grime, 1973); the shallowest recorded soil depths would only be habitable by species with high stress-tolerant attributes, and would not support the faster growth and higher nutrient demands of more competitive species. As soil depth increases, more species are tolerant of the

conditions, until competitive exclusion of the less competitive species occurs at the point of optimal soil depth. Where soil depth is generally shallow, conditions are sub-optimal for many of the more competitive species, resulting in individual plants that may be small, or less frequent, or both (Huston, 1993). In this way, the sward does not become dominated by only one or two species, and many species are able to coexist.

Overall, the relationship between species richness and soil depth proved to be complex and interactive with other factors, and would reward further analysis.

Community attributes

An understanding of the interplay of species with differing attributes would help to clarify species' successions along the soil depth gradient. Species are considered to belong to the same functional type if they use the same resources and respond to variation in the environment in a similar way (Pausas and Austin, 2001). Functional types are defined by traits such as life strategy, rooting depth, phenology, and seed size, which define their ecological niche, their contribution to ecosystem services, and their tolerance of ecological stresses (Díaz and Cabido, 2001; Díaz *et al.*, 2013). Niche differentiation is driven less by individual traits, but rather by combinations of traits (Critchley *et al.*, 2002a; Díaz *et al.*, 2013; Kraft, Godoy and Levine, 2015). Different suites of functional traits operate depending on how adaptable a species is to changing conditions, and which strategies it employs to address the associated stresses and opportunities. For example, Ravenscroft, Fridley and Grime (2014) found that *Plantago lanceolata* varied its strategy depending on soil moisture status during in-field climate change experiments, expressing drought avoidance in droughted situations and competitive strategies under control conditions. The response was modified by soil depth, with plants in shallow soils showing a greater contrast in response between drought and control plots; plants in deeper soils showed stronger associations with competitive strategies under both drought and control conditions.

Site C-S-R signatures did not support the hypothesis that deeper soil would result in an increase in competitive species (Grime C); quite the reverse, as generalised linear models indicated a significant negative association with maximum soil depth. Grime C was, however, significantly influenced by both metrics associated with variation in soil depth (range and SD), with a lower competitor fraction in the site C-S-R signature being associated with increasing heterogeneity of soil depth. The lack of significant response to soil depth or variability from the stress-tolerant and ruderal components of site C-S-R signatures reflected the general high-stress nature of the

habitat. Site C-S-R signatures also did not support the hypothesis that increased nitrogen deposition would result in an increase in competitor (Grime C) element. This is a similar result to that found by Stevens *et al.* (2010) in their work on acid grasslands along a nitrogen gradient, where C-S-R scores were not correlated with nitrogen deposition rates, and is in contrast to other studies, where competitor species have been found to increase in frequency and abundance under enhanced nitrogen deposition (Morecroft, Sellers and Lee, 1994; Emmett *et al.*, 2011).

The stress-tolerator (Grime S) element of site C-S-R signatures were expected to be associated with shallower soils and lower nutrient status, yet the overall pattern was for there to be a higher Grime S on sites with deeper soil and lower nutrient status; this held at both the mean site Grime S value and when the Grime S component of species present in individual quadrats was assessed against mean quadrat soil depth. It may be that the calculation of mean quadrat C-S-R score is an issue in this, as it was taken as the mean of all species present in a quadrat, with all species being attributed with the same abundance (species were recorded only as being present or absent, without a measure of abundance as proportion cover). Thus, the influence of more dominant species in a quadrat are not taken into account.

The relationships of overall site Grime C and Grime S to rainfall and community Ellenberg F were also contrary to expectations, with Grime C declining as community Ellenberg F increased, i.e. species with less competitive attributes were found on the wetter sites. Community Grime S was higher with higher annual precipitation than on drier sites, and also higher with higher mean annual temperature. As these grasslands overlay shallow soils and are generally well drained, droughting was considered the greater stress to be overcome, but the evidence refutes that, and indicates that stress-tolerators are better adapted to the prevailing low soil moisture levels and may be more sensitive to thermal variation. This would make them vulnerable to greater temperature variations but perhaps less vulnerable to longer summer droughts under climate change.

Looking to community attributes to help assess community responses to environmental variation, I found no significant response of community Ellenberg N to nitrogen deposition, confirming the notion that the grassland communities were not responding to an increase in soil fertility, and were not experiencing substantial eutrophication as a result. Lower values of Ellenberg L may indicate higher productivity if associated with areas of higher nitrogen deposition, and although the relationship between Ellenberg L and nitrogen was negative, it was not significant. Attention has often been on the eutrophying result of increased nitrogen input

to grassland ecosystems, particularly those considered to be well-buffered against acidification though higher soil pH. Ellenberg R values give an indication of soil pH, which, with Ellenberg R values of between 5 and 6 on all the surveyed sites, indicate moderately acid to neutral or weakly basic soils (Hill *et al.*, 1999). These Ellenberg R values may have been lower than expected for limestone grasslands, and this has implications for the conservation value of these sites. Each site was selected as being considered a good example of calcareous grassland in favourable condition according to current NE criteria, and all sites were within SSIs, which designation should have protected them from poor management practices such as fertiliser addition that are associated with a deterioration in quality of this habitat; what these designations cannot protect against is changes in large scale environmental and climatic factors.

It is widely accepted that soil acidification is associated with high or prolonged exposure to nitrogen, sulphur and other substances, and as rates of emission are expected to continue to rise (Maskell *et al.*, 2010; Smith, Schuster and Dukes, 2016), the resulting increases in nitrogen availability have potential for damaging diversity across greater areas than at present (Bobbink and Hettelingh, 2011; Bobbink *et al.*, 2012). A useful metric by which to assess acidity status of a habitat is the Ellenberg R index, which has been used to assess direction of community change in response to nitrogen and acid deposition (Newton *et al.*, 2012; Diekmann *et al.*, 2014). In their review of vegetation change on ECN sites, Rose *et al.* (2016) identified a general positive trend in Ellenberg R in response to changes in soil pH. They interpreted this as evidence that the deposition of atmospheric pollutants in the UK was sufficiently reduced that plant communities were beginning to recover from acidification caused by atmospheric pollution. I did not find a significant negative response in community Ellenberg R to nitrogen deposition; I did, however, find a significant response from Ellenberg R to mean annual rainfall, suggesting that the wetter sites were experiencing more acid conditions than sites with lower rainfall. The negative association between community Ellenberg R and deposition of other compounds likely to reduce soil pH was also noted, and though none of these were significant, they add weight to the conclusion that some of the surveyed grasslands may be under threat from the combined action of acidifying substances and climate processes.

The substrate for calcareous grasslands is necessarily alkaline, and soils are considered to be generally well-buffered against acidification (Diekmann *et al.*, 2014). Acid deposition has its strongest effect in the upper few centimetres of the soil profile, e.g. soil surface pH (in the top 3 cm) was found to be negatively correlated with soil depth at the Buxton long-term limestone grassland research site (Fridley *et al.*, 2011), as was plant-available nitrogen. On sites where soil depths were shallower, community Ellenberg R was higher, indicating a species assemblage

preferring relatively less acid conditions; conversely, deeper soils were associated with lower Ellenberg R. This suggests that deeper soils may not be offering significant buffering from acidification effects.

Climate

Mean annual temperature and mean annual precipitation were important for species richness, and accounted for most variance in species richness, though R^2 values were quite low (temperature adjusted $R^2 = 0.26$, precipitation adjusted $R^2 = 0.24$). Mean annual temperature and precipitation were not correlated with three-year averages for any of the deposition variables (N, NOx, NHy, acid or SO_2), so it was possible to differentiate their relative effects.

Both climate and deposition data were derived from interpolated gridded data presented at the 1 km scale, so it was possible to coincide the data points to that level. Maskell *et al.* (2010) identified shortcomings in the mapping of fine-scale survey data onto large-grain climate and deposition data, where they had relatively poor correlation between community metrics and the mapped data. They were using 5 km gridded data; in this study, we have used climate and deposition data at a smaller scale than that used by Maskell *et al.* (2010) but each 1 km grid potentially incorporates a number of land use types and variations in surface topography that will impact conditions at the point of survey.

Species-rich calcareous grassland is highly variable below the 1 km scale, and geographical variation between the sites was high, leading to apparent groupings of sites when particular variables were being assessed. Arnside Knott, Smardale Gill and Great Asby presented as such a group when community attributes were being considered: these three sites are geographically very close, all being west of the Pennines, and all at a similar latitude, and it is fair to suggest that there was some character of local prevailing climate that was acting to cluster them together. Removing them from the dataset made no significant difference to the results (on the remaining eight sites), but it was felt that the analysis would benefit from having the full complement of sites rather than splitting them up and losing statistical power in the process.

Nitrogen deposition

There is a high degree of variability in nitrogen emission and deposition rates are a source of concern for the accuracy of smoothing models used to generate gridded maps for nitrogen, especially for NHy, and ammonia (NH_3), as this is generally redeposited within a few hundred

metres of the emission source, and is strongly influenced by prevailing local conditions and topography (Sutton *et al.*, 1998).

There was no evidence to support a hypothesis of reduced species richness under higher nitrogen deposition rates, or to the NOx:NHy ratio, which agreed with the results of other field survey studies into the effects of nitrogen deposition on calcareous grasslands, e.g. Maskell *et al.* (2010). There was similarly no species richness response to nitrogen by form – species richness appeared resistant to the effects of any of the deposition variables. This was not unexpected, as calcareous grasslands are typically not very responsive to nitrogen inputs – this is partly due to the low-nutrient adaptation characteristic of many grassland species, and to phosphorus colimitation, which prevents additional plant-available nitrogen being accessed and used to increase productivity. Where nitrogen deposits are not taken up by plants, the excess (as nitrate) is leached from the soil, leaving an accumulation of hydrogen ions, acidifying the soil. The critical load for calcareous grassland was set in 2002 as 15-25 kg N ha^{-1} yr^{-1} (Bobbink *et al.*, 2003), and later revised to 20 kg N ha^{-1} yr^{-1} for UK calcareous grasslands, referencing field and experiment observations of ecosystem changes (Emmett *et al.*, 2011; Hall *et al.*, 2011). Lower critical loads were set for more sensitive subcommunities within the broad calcareous grassland classification, e.g. rock ledge and bryophyte/lichen-rich communities (Bobbink and Hettelingh, 2011) have a critical load of 5-10 kg N ha^{-1} yr^{-1}. All sites barring one (Smardale Gill) had total nitrogen deposition rates above the lower limit of the current critical load; two sites were also outside the upper limit, suggesting that at least those two sites were at risk of significant damage from N deposition, and that all sites had heightened potential for such damage.

Sites were selected from the Natural England database of calcareous grassland SSSIs in favourable condition, to reduce possible confounding factors and to allow the sites to be as comparable as possible. Site condition assessment is not very sensitive to nitrogen impacts, so there is potential for such impacts to be present even on sites in favourable conditions. The sample size is, however, small at eleven sites, and with only two sites experiencing atmospheric nitrogen deposition above the upper limit of the current critical load, it may not be large enough to detect an impact, i.e. one potential limitation of using sites in favourable condition is that management may be offsetting nitrogen-deposition impacts.

Summary

Of the soil metrics considered, maximum rather than mean depth proved to have most influence on species richness. The finer-scale responses to soil depth metrics, and the association of increasing soil depth heterogeneity (as expressed by soil depth standard error) with increasing species richness, add support to the general recognition elsewhere that small-scale habitat heterogeneity may be important for community organisation in calcareous grasslands (Fitter, 1982; Fridley *et al.*, 2011).

There was some evidence of acidification in the wetter calcareous grasslands surveyed, though it is not possible to gauge current rates and direction without considering if and how the communities change over time, which would require a revisiting of the sites in order to gather further time-point data. There was little evidence of eutrophication, and low frequency of highly competitive species; this was considered to be due to phosphorus limitation restricting the widespread advance of more competitive species rather than a lack of nitrogen input into the system. Soil depth did not appear to be ameliorating the effect of nitrogen deposition or climate with respect to species richness, but the degree of heterogeneity in soil depth was found to modify species richness responses to variation in temperature and precipitation.

Chapter 5 Future prospects for calcareous grasslands under climate change

In this chapter, I present my main findings and consider what they mean for the future of calcareous grasslands in the face of climate change.

For this thesis, I have undertaken three data-collection exercises, using three different approaches, at three different spatial and temporal scales. These methods, themes and scales are set out in two infographics to illustrate cross-study connectivity.

5.1 Summary of approach

The overarching research question behind this thesis is how projected changes in UK climate may affect the plant community composition of lowland calcareous grasslands. In order to investigate this, I have used three approaches at different spatial scales: the landscape or ecosystem grassland survey, the open field experiment, and the mesocosm experiment.

Briefly, the three approaches are:

- a geographically diverse survey of calcareous grasslands to investigate how soil depth properties, climate variables and atmospheric nitrogen deposition interact to influence the species and functional composition of the surveyed plant communities.

- a field-based rainfall manipulation experiment investigating the interactive effect of variations in soil moisture and increased nitrogen availability on productivity and functional composition;

- an outdoor mesocosm experiment designed to investigate how soil depth interacts with nitrogen form and increased availability to affect productivity and resource allocation in a model grassland community;

The response of ecosystems and their components to environmental conditions is expressed differently depending on the time and spatial scales within which we observe those responses (Kratz *et al.*, 2003; Smith, Knapp and Collins, 2009). The three lines of investigation reported in this thesis were not only carried out at different spatial scales, but also within differing timescales:

- the geographical survey data presents a snapshot of the vegetation communities at eleven sites across the country taken in May 2018;

- data from the field-based rainfall manipulation at RainDrop extended over three years (including the baseline surveys in 2016), and has potential to continue for many years;

- the mesocosm experiment ran over two growing seasons.

There is also a response hierarchy at work: individual plant responses to changing conditions are the most immediate, and will reflect changes over a short timescale through, for example, growth rates and variation in primary productivity (as seen in the mesocosm experiment and in the RainDrop data). Longer term responses to changing environmental conditions are reflected

in the wider local community, such as under the rainfall manipulations at RainDrop, with expansion and contraction of species' abundances. Changes at an ecosystem level take decades to be expressed, particularly in terms of taxonomic or functional diversity as a result of invasion and local extinctions as species adapt or otherwise to wider environmental change. The multiscalar approach taken throughout this thesis allows different questions to be posed depending on the scale of investigation. The hierarchy of response to variation in certain environmental conditions at spatial and temporal scales, along with the related hypotheses that were tested, is summarised in Figure 5.1.

At its most fundamental, the best way to see how an ecosystem changes over time is to observe it over that time. This is a purist approach, but requires time and resources, and does not allow for predictions to be made concerning the direction and magnitude of responses to changing environmental conditions. Experimental approaches harness the need for pragmatism with the possibility of modelling or prediction-making. Each of the approaches used in this thesis carries advantages and disadvantages in the collection and interpretation of the data.

Mesocosm experiments are useful in that they allow for greater control of conditions and community membership, and can produce useful data in a short timescale and with minimum effort beyond the initial setting up. As an in-field experiment on an existing local community, RainDrop has potential to continue to produce useful data for many years to come; indeed, as time goes on, the data from RainDrop will become more usable as a potential predictor of responses in the wider calcareous grassland ecosystem, as the confounding effects of the change of management become more remote in time.

Although the snapshot approach of the landscape-scale investigation does not allow for useful comments to be made on the trajectories taken by plant communities on individual sites to their current position, they provide a useful starting point for consideration of how the ecosystem may develop in the face of changing climatic conditions – a plant community is a reflection of the average conditions on site over time. As such, that average of conditions may allow for the application of "space for time" substitution (SFT), whereby current spatial distributions of species are used as correlates for temporal scenarios (Blois *et al.*, 2013; Metz and Tielbörger, 2016).

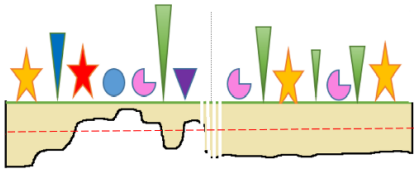
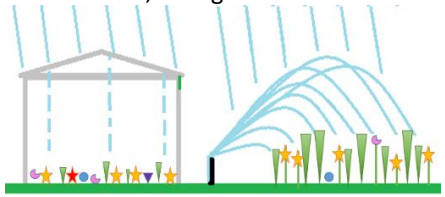
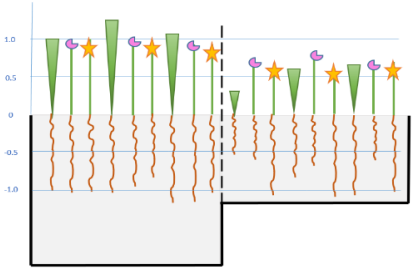
scales *spatial*	*time*	**investigation** and associated themes	**hierarchical response**	**hypotheses**	
ecosystem	*decade*	**grassland surveys** rainfall, nitrogen, soil metrics 	• loss of species due to competitive interactions and tolerance limits • immigration of species more suited to the new conditions	H4.1, H4.2	Plant species richness and diversity will decline with increasing soil depth.
				H4.3	Species richness will increase with greater soil depth heterogeneity.
				H4.4	Plant communities on shallower soils will have a higher stress-tolerant element in site CSR signatures.
				H4.5	Proportion of competitive species will be greater on sites with a) higher rainfall, b) higher N deposition rates, or c) deeper soil.
				H4.6	Soil depth will modify response to other environmental variables.
local community		**RainDrop** soil moisture, nitrogen 	• reordering of species' rank abundance as some species are advantaged by changes in environmental conditions	H2.1	Diversity will be higher drought plots than in irrigated plots.
				H2.2	Grass to forb abundance ratio will increase in irrigated plots compared to that in drought plots.
				H2.3	More competitive species will be more abundant in irrigated plots compared to the drought plots.
				H2.4	Reduction in above-ground biomass in drought plots will be proportionally similar to an increase in above-ground biomass in irrigated plots.
				H2.5	Plant biomass will increase with nitrogen addition.
		mesocosms nitrogen, soil depth 	• individual plant responses, e.g. resource acquisition and utilisation leading to changes in above- and below-ground biomass	H3.1	Biomass will be greater in deep soil.
				H3.2	Species biomass will increase with nitrogen enrichment.
				H3.3	Species biomass will show a stronger positive response to oxidised nitrogen than to reduced nitrogen enrichments.
				H3.4	The root:shoot ratio will decrease with an increase in soil nitrogen and water availability.
individual	*season*			H3.5	Tissue nitrogen content will be greater under nitrogen enrichment.

Figure 5.1 Hierarchical scale of community responses, after Smith, Knapp and Collins, (2009) and Collins et al. (2014).

SFT is particularly useful for modelling community composition and species turnover as changes in composition have been found to strongly correlate with climate variations along temporal and spatial gradients (Blois *et al.*, 2013; Collins *et al.*, 2014), although there are caveats to using such proxy systems, primarily around the confounding interactions of plant species abundances with environmental variables such as temperature, precipitation and soil properties (Metz and Tielbörger, 2016). A major advantage of SFT is that it allows studies to be undertaken within a single season, and so remains a popular approach for practical and economic reasons. Critics of SFT suggest that ecosystem responses are influenced by a multitude of factors beyond the driver of interest, and that spatial variation in such drivers, e.g. annual precipitation, can lead to misleading results (Walker *et al.*, 2010; Banet and Trexler, 2013). Walker *et al.* (2013) also considered SFT to be inappropriate where diversity is high, as it typically is on calcareous grasslands. Used mindfully, the space-for-time approach could be used to infer the direction and magnitude of biodiversity changes from contemporary spatial patterns.

5.2 Themes

Two well-documented drivers of change in plant communities relate to the availability of moisture and nutrients. In this thesis, I have also considered the role of soil depth in two of the data chapters. Soil depth is a generally overlooked yet fundamental aspect of the environment that impacts on soil moisture and nutrient availability and has potential to mediate the effect of variation in rainfall and atmospheric nitrogen deposition rates.

The three central themes, therefore, are:

- soil moisture – Chapter 2 covers the rainfall manipulation treatments applied as part of the RainDrop experiment; in chapter 3 (mesocosm experiment), soil depth is used as a proxy for available soil moisture, in that greater depth provides a greater potential reservoir; chapter 4 considers the influence and interactions of mean annual rainfall with a varying soil reservoir at the landscape level.

- soil nutrient availability, with particular reference to nitrogen as the most important plant nutrient; taking this approach ties in with environmental pollution and management issues, as it could be derived from agricultural application or via atmospheric deposition. Nitrogen addition was carried out for the open field experiments in chapter 2 and the mesocosm experiment in chapter 3; mean annual atmospheric nitrogen deposition rates were included as a fixed effect for the sites studied in chapter 4.

- soil depth – soil depth set at two fixed depths in the mesocosm experiment (chapter 3). The most detailed study of soil depth was made as part of the landscape survey detailed in chapter 4, where a number of metrics were calculated from those measurements (e.g. mean quadrat soil depth, mean site soil depth, minimum and maximum site soil depth, range of site soil depth (i.e. maximum - minimum depths).

The two lower-level investigations each directly consider two factors as potential drivers of change which then feed up the hierarchy into the landscape/ecosystem level (grassland surveys), where all three main themes are considered (Figure 5.2):

- individual level (mesocosm experiment) – soil depth, nitrogen availability;
- local community level (RainDrop) – rainfall manipulation, nitrogen availability,

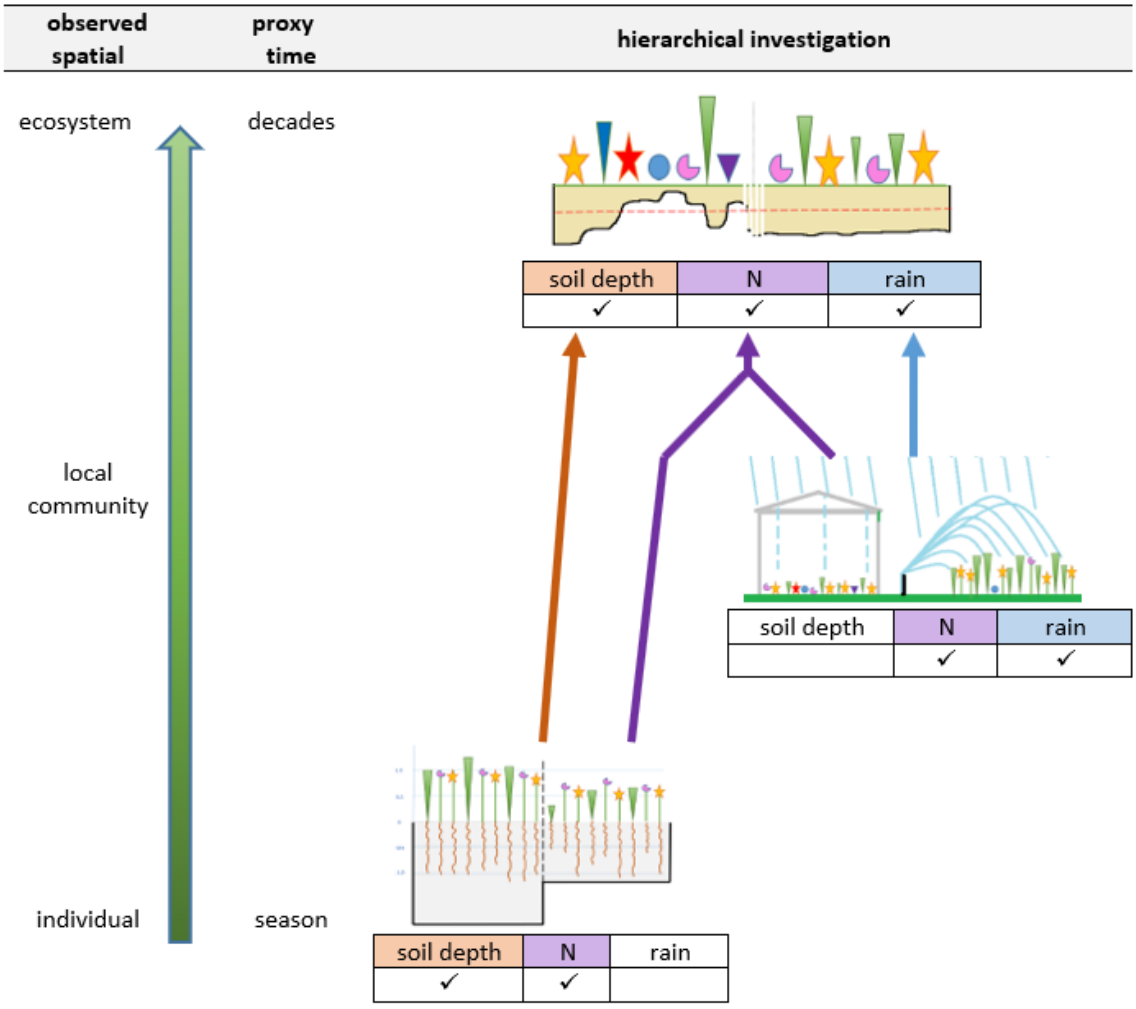


Figure 5.2 Schematic: how investigative themes link across spatial hierarchy.

The main findings are summarised in Table 5.1, below.

Table 5.1 Thematic summary of main findings.

theme	spatial scale	hypotheses		results
soil moisture	ecosystem	H4.5a	Plant communities on sites with higher rainfall will contain a greater proportion of competitive species.	Not supported – higher values of Grime C were not significantly associated with higher mean annual or summer rainfall.
		H2.1	Diversity will show a negative correlation with received rainfall amount, i.e. will be higher under the drought treatment.	Supported – Simpson's Index of Diversity was higher under drought treatment than under the irrigated treatment, in both 2017 and 2018.
	local community	H2.2	Grass to forb abundance ratio will increase under enhanced rainfall treatment compared to that under the drought treatment	Supported – grass:forb ratio in 2018 was higher in irrigated plots compared with drought plots, across all nitrogen treatments.
		H2.3	More competitive species will increase in abundance under enhanced rainfall treatment compared to the drought treatment.	Supported – Grime C fraction was higher on the irrigated plots than on the drought plots, in both experimental years.
	individual	H2.4	Reduction in above-ground biomass under the drought treatment will be proportionally similar to an increase in above-ground biomass under the enhanced precipitation treatment.	Not supported - net change in total biomass compared with that in ambient plots was proportionately greater in drought plots in both experimental years.
	Summary – soil moisture • Open-field experiment and mesocosm study showed that soil moisture availability significantly influenced both above and below ground productivity (chapters 2 and 3).			

	• Biomass data from the open-field experiment highlighted the importance of seasonality of rainfall to productivity, which confirmed the potential for climate change to disturb these grassland communities through variation in both timing and magnitude of rainfall events (chapter 2). • An inverse relationship exists between species richness and mean annual rainfall on calcareous grasslands, due to a relative decline in herbaceous forb and legume species, and a corresponding increase in dominance by graminoid species on wetter sites (chapter 4). • Community attributes are significantly influenced by precipitation – community Ellenberg N increased with increasing precipitation; community Ellenberg R declined with increasing precipitation, indicating an increase in acidity on wetter sites (chapter 4). • Community composition was influenced by mean annual temperature, with a lower number of perennial species and a greater number of annuals on warmer sites. Forb and legume richness were both significantly higher on warmer sites; the increase was significant for the annual species of both these plant groups (chapter 4).			
nutrient availability	ecosystem	H4.5b	Plant communities on sites with higher N deposition rates will contain a greater proportion of competitive species.	Mixed results – the Grime C fraction of site C-S-R signatures declined significantly with increasing total N deposition rates; NOx and NHy deposition did not in themselves significantly influence Grime C, but the NOx/NHy ratio did, whereby higher Grime C fractions were associated with higher NOx/NHy ratios.
	local community	H2.5	Biomass will increase in response to nitrogen addition.	Mixed responses; in drought year 2017, total biomass was measurably but not significantly greater in both nitrogen-addition plots cf control under the irrigated and procedural control treatments. In 2018, both nitrogen addition treatments resulted in greater total biomass in the ambient control plots; under the irrigation and drought treatments, the water-addition control produced more biomass than either nitrogen addition treatment. .

	individual	H3.2	Biomass will increase with nitrogen enrichment.	Mixed responses that were observed but generally not significant, and changed depending on N form and soil depth. Total mesocosm (community) biomass was greater for both N forms in shallow soil, and for Nred in deep soil. Species' total biomass changed depending on N form and soil depth, apart from *Dactylis*, which was more productive with nitrogen addition in both soil depths. *Lotus* total biomass was lower with both N forms in deep soil, and greater with both N forms in shallow soil. *Silene* total biomass was lower than in the controls with NOx in deep soil, and with Nred in shallow soil.
		H3.3	Biomass will show a stronger positive response to oxidised nitrogen than to reduced nitrogen enrichments.	Not fully supported – there was no consistently positive response to N addition for either NOx or Nred. Where the N-addition responses were both positive, the NOx response was not consistently greater than the Nred response. Individual species' responses varied depending on N form and soil depth.
		H3.4	The root/shoot ratio will decrease with a) deeper soil, and b) nitrogen addition.	Mixed responses, which varied by species. All species' root/shoot ratios were most strongly influenced by soil depth (H3.4a). No species was significantly influenced by N addition (H3.4b), or by the interaction of N and soil depth.
		H3.5	Tissue nitrogen content will be greater in nitrogen-enriched conditions.	Response varied by species, and depended on N form and soil depth. Shoot N was reduced in all species with Nred addition in deep soil, compared with the controls. *Lotus* and *Dactylis* shoot N were greater with NOx in deep soil; *Silene* shoot N was lower with both N forms in deep soil. In shallow soil,

				Lotus shoot N was lower with both N forms; *Dactylis* shoot N was greater with both N forms. All species' root N was lower than controls with both N forms in shallow soil, and varied by species in deep soil.

Summary – nutrient availability

- Species richness on surveyed sites was not significantly influenced by nitrogen deposition rates (chapter 4). This is contrary to other large-scale surveys along N gradients, e.g. Stevens et al 2004, and many other studies that showed a decline in diversity as N increases.

- There was little response of biomass to nitrogen deposition, suggesting that the influence of soil moisture far outweighed the effect of increased nitrogen/nutrient availability (chapters 2 and 3).

- The competitive component of survey site C-S-R signatures was reduced by increasing total nitrogen deposition, though there was no significant difference between the C-S-R response to reduced or oxidised nitrogen (chapter 4). This is against expectation, which was that there would be an increase in more competitive species / a decrease in abundance of stress-tolerators.

- Species identity was important in the response to nitrogen form (mesocosm experiment, chapter 3); individual species' allocation of nutrients was very plastic and influenced by an interaction with soil depth. In deep soil, all species increased proportional allocation to shoot biomass with additional oxidised nitrogen availability, and reduced shoot biomass allocation with additional reduced nitrogen availability, compare with the controls. This indicated an actively inhibiting effect through increased NHy (chapter 4).

- There was some evidence for acidification on some sites as site Ellenberg R showed a negative trend with precipitation, i.e. wetter sites were more acidic (chapter 4). This may be an effect of increased deposition of acidifying substances in rainfall, and also an increased leaching of base cations leading to an accumulation of H^+ in the soil; more acid-tolerant vegetation is commonly found in northern UK as more acid soils overlie underlying basic rocks due to this effect of higher rainfall.

	• There was little evidence for eutrophication as Ellenberg N was not significantly correlated with higher nitrogen deposition (chapter 4).			
soil depth	ecosystem	H4.1	Plant species richness will decline with increasing soil depth.	Not supported – site species richness increased with an increase in value for all soil depth metrics; significantly so for increasing maximum soil depth, and soil depth range. Quadrat species richness declined with increasing soil depth metrics, but not significantly so.
		H4.2	Plant communities will be more diverse on shallower soils.	Not supported – Simpson's Index of Diversity increased with increasing soil depth metrics, i.e. site communities were more diverse on sites with deeper soil.
		H4.3	Species richness will increase with greater soil depth heterogeneity.	Mostly supported – site species richness was significantly higher on sites with a wider range of soil depths; this pattern was also seen at quadrat level, though not significantly so.
		H4.4	Plant communities on shallower soils will have a higher stress-tolerant element in site CSR signatures.	Mean, range and soil depth SD soil metrics were all positively associated with Grime S, but not significantly so. At quadrat level, Grime S declined with an increase in all soil depth metrics (significantly for minimum, maximum and mean soil depths).
		H4.5c	Plant communities on sites with deeper soil profiles will contain a greater proportion of competitive species.	Not supported – site Grime C declined with increasing maximum, mean, range and soil depth SD (significantly so for maximum, range and SD), i.e. higher Grime C fractions in site C-S-R signatures were associated with shallower or less variable soil depth metrics.

		H4.6	Soil depth will modify species richness response to other environmental variables.	Supported - the data suggest some dependency of species richness on the level of soil depth metrics, particularly with regard to maximum, range and soil depth SD.
	local community	H3.1a	Total mesocosm biomass will show a positive relationship with soil depth.	Supported – total community (mesocosm) was higher in the deep soil treatments compared with control and shallow soils; community shoot and root biomass were also greater in deep soil.
	individual	H3.1b	Species biomass will show a positive relationship with soil depth.	Mostly supported – total biomass production was greater in deep soil for all species, as was shoot biomass. Root biomass was greater in deep soil for *Dactylis* and *Silene*, but not for *Lotus*.

Summary – soil depth

- Species richness was significantly influenced by soil depth range; sites with a greater range (maximum – minimum measured depth) had higher species counts (chapter 4). This supported the findings of Fridley *et al.* (2011), that heterogeneity in soil depth was a positive influence on species richness in the calcareous grassland at the Buxton Climate Change Impacts Laboratory, Derbyshire.

- Increased soil moisture and nutrient availability provided by deeper soil renders some species vulnerable to scorching under more extreme summer temperatures; this was observed in the deep soil mesocosms, where lower root:shoot ratios resulted in above-ground biomass experiencing excess water loss, with subsequent tissue damage (chapter 3).

- The competitor component of site C-S-R signatures declined with greater soil depth (chapter 4).

- Resource allocation is influenced by soil depth; root:shoot and C:N ratios both showed a strong signal in response to soil depth. The results from the mesocosm experiment (chapter 3) indicated that soil depth is positively correlated with biomass production, though the mechanisms responsible were unclear.

	Interactive role of soil depth in modifying species richness responses • Mean soil depth was not itself a significant predictor of species richness, but interacted with other variables to modify species richness response to mean annual and mean summer temperatures; species richness increased with increasing mean temperature at a faster rate where soil was deep (chapter 4). • Site-level soil depth variability increased species richness responses to temperature and precipitation; species richness was higher in more variable soils, and also increased with increasing temperature at a faster rate than sites with less variable soil depth (chapter 4). • Mean soil depth is therefore an important factor when considering temperature rises under climate change, and variation in soil depth is also an important factor when considering species richness and productivity responses to changes to rainfall patterns under climate change (chapters 3 and 4).

5.3 Robustness of synthesised evidence

There are limitations imposed by the spatial and temporal scales used in these investigations, which should be borne in mind when considering the potential for drawing predictions from the results. The short duration of the mesocosm experiment and the study at RainDrop restrict potential for extrapolating beyond the very close future. The value of the RainDrop data lies, perhaps, more in its being the baseline data for what is hopefully a long-running experiment, where the drought and irrigation treatments continue to be imposed and to affect the underlying localised plant community in the experimental plots.

Both RainDrop and the mesocosm experiments suffered from recent disturbance events: for the mesocosms, this was in the form of the setting-up of the experiment, and for RainDrop, the construction of the rainshelters and associated irrigation works. RainDrop also suffered a change in land management, whereby it moved from being sheep-grazed to a mowing regime, with concomitant changes in nutrient cycling and disturbance cycles. In the longer term, the effect of this change will be reduced. Both the mesocosms and RainDrop also experienced atypical climate events in the form of extreme and protracted high summer temperatures, which not only led to prolonged drought conditions, but also to scorching of the above-ground vegetation. Over time, the acute effect of such events will be diminished as they become part of a longer time average of conditions, but for studies lasting two or three growing seasons, such events have high potential to skew results in the short term. Such annual variations in environmental variables may be reflected in snapshot surveys, but site characteristics are essentially reflections of average conditions - even in a year with atypically low rainfall, for instance, the vegetation of a wet habitat will still exhibit species and characteristics of a wet site (Laurenroth and Sala, 1992).

Plant communities considered at the local community or ecosystem level are considered to have reached a degree of equilibrium with local conditions, which will take time to change in the face of, for example, changing rainfall patterns. Such variations in climate variables will have influence at both the short and the long term, depending on the metrics being investigated, and the timescale at which those metrics operate (Myster and Malahy, 2008; Smith, Knapp and Collins, 2009; Walker *et al.*, 2010). In the short term, for example, increased water availability at RainDrop resulted in an increase in above-ground biomass, in the same way that both above- and below-ground biomass was greater in the deep soil mesocosms. In this respect, a general conclusion can be drawn from the mesocosm and RainDrop experiments that can be scaled-up to landscape or ecosystem level, i.e. that increased soil moisture leads to an

increase in productivity in calcareous grasslands. Processes that drive changes in species richness or diversity operate on longer timescales; invasion and extinction processes operate over decades rather than seasons. For example, although higher mean annual rainfall was associated with lower species richness at the ecosystem scale, there was no significant difference in species richness between the irrigated and the drought plots at RainDrop after two years of imposed rainfall manipulation – though there is an expectation that species richness will change over time in response to the changing soil moisture conditions. In the medium term, the experimental plots at RainDrop did exhibit changes in abundance if not in species richness, as was reflected in the Simpson's index of diversity.

5.4 Integrating community responses in the context of climate change

The Earth's climate is irrefutably changing, and at an increasing rate; it is no longer a question of if, or even when, but rather what has been the duration, and by how much will ecosystems be altered by what is predominantly human activity. The impacts of climate change operate on all scales, and on many processes beyond the widely acknowledged increases in surface and sea temperatures; alterations in temperature feed into other Earth systems such as environmental chemistry and hydrology, which in turn cascades the effect of change into ecological responses. Predicting ecological responses to climate change requires a range of approaches to help unpick complex mechanistic relationships between communities and their environments (Stewart *et al.*, 2013). Water availability is a major factor in plant growth and development, so changes to the amount and seasonality of precipitation have potential to have significant impact on terrestrial ecosystems.

Acting in concert with climate change are other factors that significantly influence ecosystems, notably nitrogen deposition, and many studies have been aimed at interpreting ecosystem responses to both deposition rate and accumulation over time (Chapin, 1980; Stevens *et al.*, 2006, 2009; Maskell *et al.*, 2010; Field *et al.*, 2014; Ma *et al.*, 2016; Wilkins, Aherne and Bleasdale, 2016). Many of these studies considered total nitrogen deposition, with the underlying assumption that the concentrations and deposition of NOx and NHy were approximately equal. A growing awareness of the differences between NOx and NHy sources on residence times, deposition patterns, and influences on plant communities, have led to a reassessment of the influence of the different forms of nitrogen. Following on from the work of Fridley *et al.* (2011) on soil depth heterogeneity, I decided to also investigate the influence of soil depth

Calcareous grassland communities are characteristically species rich, and composed of slow-growing, stress-tolerant species, whose presence on a site is a central element of assessing the quality of the community in terms of biodiversity value (Wilson and Wheeler, 2016). Typically, they experience extremes of climate, and typical species are tolerant of low nutrient, often droughted conditions. Resistance to changes in these conditions can be assessed through platforms such as RainDrop or via mesocosm experiments, and community traits linked to productivity readily measured. To investigate resilience requires a longer study, in order to satisfy the time component inherent in the definition, and to assess how long the system takes to return to its starting position. For this, long-term open field experiments are ideally suited, though the application of modelling approaches such as space for time substitution have potential to provide meaningful results in the shorter term than a purely observation-based approach.

Field surveys allow for snapshots of communities to be linked to prevailing environmental conditions, and traits such as tolerance of particular nutrient or soil moisture regimes can be used to measure responses to those conditions. As stated by Hirst *et al.* (2005), the determination of resilience depends on which aspect of an ecosystem is under investigation. In terms of the main environmental factors considered in this thesis, established calcareous grassland appears to be both resistant and resilient. At the Buxton Climate Change Impacts Laboratory, most species did not exhibit large changes in abundance in response to 20 years of temperature and rainfall manipulation (Grime *et al.*, 2000; Fridley *et al.*, 2011; Ravenscroft, Fridley and Grime, 2014), and high nitrogen inputs of 140 kg N ha^{-1} yr^{-1} to a grazed calcareous grassland had no significant effect on either species growth or community composition (Morecroft, Sellers and Lee, 1994).

Grasslands have been described as the ecosystem most sensitive to water availability (Huete, 2016; Seddon *et al.*, 2016), a concept that finds support in the species richness response to precipitation of the survey sites, and productivity responses in the two experimental investigations undertaken as part of this thesis. To measure resilience in terms of species richness is to consider only one aspect of an ecosystem, with the concomitant weaknesses inherent in such a simplification. Through processes of reassembly and species turnover following environmental perturbation, species richness may rebound to levels close to that prior to disturbance, but species composition may be altered (Critchley *et al.*, 2002b; Zhang *et al.*, 2017), and ecosystem stability become more overtly dependent on functional rather than species diversity (Helsen, Hermy and Honnay, 2012; Debouk, De Bello and Sebastia, 2015).

The work undertaken in this thesis has shown that interactions between environmental factors are important predictors of species richness and productivity, and that community diversity depends to a large extent on habitat heterogeneity. Most calcareous grassland species are highly stress tolerant; by extension, therefore communities composed of such species will also be highly tolerant of particular stresses. The vulnerability of these communities to climate change will likely be in their more competitive and ruderal members.

The work for this thesis has also shown that soil depth and soil depth heterogeneity play an important role in maintaining species and functional richness on calcareous grasslands, and that future community compositions will be influenced by the interaction of soil depth with other climatic and depositional factors.

5.5 Suggestions for further work

Site management was not considered during the surveys, though this has been shown to influence species and functional composition (Wilson, Wells and Sparks, 1995; Burke and Grime, 1996; Hunt, Colasanti and Hodgson, 1996; von Felten *et al.*, 2009). There were general changes observed in the vegetation across the RainDrop site that were not associated with the treatments and were considered to be a result of the change in management from sheep grazed to a biannual mowing regime. For conservation and restoration purposes, an understanding of the influence and interaction of practices with prevailing and projected climate is critical to developing appropriate management plans for this habitat.

Although there were no significant correlations between grass:herb ratio and any of the environmental variables at the eleven survey sites, the grass:herb ratio may be a useful metric for monitoring change in calcareous grassland communities through revisiting at these sites in the future.

Appendix 1 Ellenberg Indicator Values (EIVs)

Definitions of values adapted for British and Irish flora, as given in Hill *et al.* (1999)

A1.1 Ellenberg indicator definitions for moisture (F)

EIV	Explanation
1	Indicator of extreme dryness, restricted to soils that often dry out for some time.
2	Between 1 and 3.
3	Dry-site indicator, more often found on dry ground than in moist places.
4	Between 3 and 5.
5	Moist-site indicator; mainly on fresh soils of average dampness.
6	Between 5 and 6.
7	Dampness indicator; mainly on constantly moist or damp, but not on wet soils.
8	between 7 and 9.
9	Wet site indicator; often on water-saturated, badly aerated soils.
10	Indicator of shallow-water sites that may lack standing water for extensive periods.
11	Plant rooting under water, but at least for a time exposed above, or plant floating on the surface.
12	Submerged plant, permanently or almost constantly under water.

A1.2 Ellenberg indicator definitions for nutrient level (N)

EIV	Explanation
1	Indicator of extremely infertile sites.
2	Between 1 and 3.
3	Indicator of more or less infertile sites.
4	Between 3 and 5.
5	Indicator of sites of intermediate fertility.
6	between 5 and 7.
7	Plants often found in richly fertile places.
8	Between 7 and 9.
9	Indicator of extremely rich situations, such as cattle resting places or near polluted rivers.

A1.3 Ellenberg indicator definitions for reaction (R)

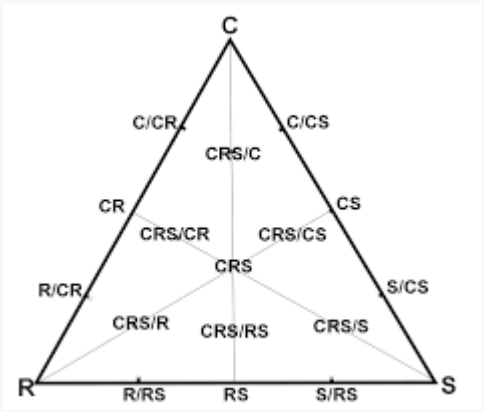
EIV	Explanation
1	Extreme acidity, never found on weakly acid or basic soils.
2	Between 1 and 3.
3	Mainly on acid soils, but exceptionally also on nearly neutral ones.
4	Between 3 and 5.
5	Moderately acid soils, only occasionally on very acid or on neutral to basic soils.
6	Between 5 and 7.
7	Weakly acid to weakly basic; never on very acid soils.
8	Between 7 and 9.
9	Basic reaction; always on calcareous or other high-pH soils.

A1.4 Ellenberg indicator definitions for light (L)

EIV	Explanation
1	Plant in deep shade (no examples in Britain or Ireland).
2	Between 1 and 3.
3	Shade plant, mostly less than 5% relative illumination, seldom more than 30% illumination when trees are in full leaf.
4	between 3 and 5.
5	Semi-shade plant, rarely in full light, but generally with more than 10% relative illumination when trees are in leaf.
6	between 5 and 7.
7	Plant generally in well-lit places, but also occurring in partial shade.
8	Light-loving plant rarely found where relative illumination in summer is less than 40%.
9	Plant in full light, found mostly in full sun.

Appendix 2 RainDrop species information

A2.1 Simplified CSR classes



Grime's CSR classes (from Hancock (2016))

Grime's triaxial representation of plant strategies can be subdivided from the three primary strategies (C, competitors; S, stress-tolerators; R, ruderals) into secondary and tertiary levels. Each class is defined by the relative levels of the three primary factors, as plotted along the axes.

Where CSR classes were amalgamated, classes were contracted as follows:

CSR class	secondary CSR
C/CR	C
C/CSR	C
C/SC	C
CR	CR
CR/CSR	CR
CSR	CSR
R	R
R/CR	R
R/CSR	R
R/SR	R
S	S
S/CSR	S
S/SC	S
SC	SC
SC/CSR	SC
SR	SR
SR/CSR	SR

A2.2 Plant group, CSR class, Ellenberg Indicator Values and life history information for species encountered in RainDrop survey quadrats.

Species identification follows Rose and O'Reilly (2006) and Stace (2010) for forbs, legumes and woody species; grasses follow Hubbard (1984). Life history data was taken from the BRC Atlas of British and Irish Flora (https://www.brc.ac.uk/plantatlas/), apart from Ellenberg Indicator values (EIV) for *Galium pumilum, Brachypodium rupestre,* and *Phleum bertolinii*, for which data was taken from the Ecological Flora of the British Isles database (Fitter and Peat,(1994), http://ecoflora.org.uk/).

Grime's C-S-R strategies are associated with degrees of competitiveness (Grime C), stress-tolerance (Grime S) and ruderality (Grime R). CSR strategy data was taken from the UCPE online tool developed by Sheffield University (Hunt *et al.*, 2004) apart from the following species, that were not found in the UCPE database: Pierce *et al. (*2017) for *Bromus commutatus* and *Vicia sativa*; Dennis (2012) for *Phleum bertolinii*; *Galium pumilum*, which uses the strategy allocated by Riibak *et al.* (2015).

Ranunculus parvoflora (assigned R) and *Orobanche minor* (broomrape species, assigned S) were assigned as a result of literature search, though no definitive strategy was found. *Hieracium* species (not including *Pilosella*), *Prunus, Quercus* and *Rosa* species do not have detailed habitat or life form information, as they were only reliably identified to genus.

Key:	C-S-R	Grime's C-S-R classes; source as above.
	species	as per Rose, Stace or Hubbard, as above.
	plant group	grass: graminoids, i.e. grass, sedge, wood rush species.
		forb: non-leguminous forb species.
		legume: leguminous forb species.
		woody: *Rubus, Rosa* and tree species.
	EF	EIV for sensitivity to moisture.
	EL	EIV for sensitivity to light.
	EN	EIV for sensitivity to nutrient levels.
	ER	EIV for sensitivity to soil pH.
	life history	perennation class – annual, biennial, perennial.

Note: see Appendix 1 for Ellenberg Indicator definitions.

RainDrop species information

CSR class	species	plant group	EL	EF	ER	EN	life history
CSR	*Agrostis capillaris*	grass	6	5	4	4	per
CR	*Agrostis stolonifera*	grass	7	6	7	6	per
SR/CSR	*Anthoxanthum odoratum*	grass	7	6	4	3	per
C/CSR	*Arrhenatherum elatius*	grass	7	5	7	7	per
SC	*Brachypodium pinnatum*	grass	7	3	8	3	per
SC/CSR	*Brachypodium sylvaticum*	grass	6	5	6	5	per
R/CSR	*Bromus commutatus*	grass	7	4	7	2	ann
SC/CSR	*Bromus erectus*	grass	7	4	8	3	per
R/CR	*Bromus hordeaceus*	grass	8	4	7	4	ann
R/CR	*Bromus sterilis*	grass	8	4	8	7	ann
S/CSR	*Carex caryophyllea*	grass	7	4	7	2	per
S/SC	*Carex flacca*	grass	7	5	6	2	per
S/CSR	*Carex sylvatica*	grass	4	5	6	5	per
R/CSR	*Cynosurus cristatus*	grass	7	5	6	4	per
C/CSR	*Dactylis glomerata*	grass	7	5	7	6	per
SC/CSR	*Festuca arundinacea*	grass	8	6	7	6	per
S	*Festuca ovina*	grass	7	5	4	2	per
CSR	*Festuca rubra*	grass	8	5	6	5	per
SC/CSR	*Helictotrichon pratense*	grass	7	4	7	2	per
S/CSR	*Helictotrichon pubescens*	grass	7	4	7	3	per
CSR	*Holcus lanatus*	grass	7	6	6	5	per
R/CR	*Lolium multiflorum*	grass	7	5	7	7	ann
CR/CSR	*Lolium perenne*	grass	8	5	6	6	per
S/CSR	*Luzula campestris*	grass	7	4	5	2	per
SR/CSR	*Phleum bertolinii*	grass	8	4	7	4	per
R	*Poa annua*	grass	7	5	6	7	ann
CSR	*Poa pratensis*	grass	7	5	6	5	per
R/CSR	*Poa trivialis*	grass	7	6	6	6	per
CSR	*Trisetum flavescens*	grass	7	4	7	4	per
CSR	*Achillea millefolium*	forb	7	5	6	4	per
CSR	*Agrimonia eupatoria*	forb	7	4	7	4	per
SR/CSR	*Anacamptis pyramidalis*	forb	8	4	8	3	per
C/CR	*Anthriscus sylvestris*	forb	6	5	7	7	per
S	*Broomrape sp*	forb	7	4	8	6	unk
CSR	*Centaurea nigra*	forb	7	5	6	5	per
SC/CSR	*Centaurea scabiosa*	forb	8	3	8	3	per
R/CSR	*Cerastium fontanum*	forb	7	5	5	4	per
R/SR	*Cerastium glomeratum*	forb	8	3	8	3	ann
R/CSR	*Cirsium eriophorum*	forb	8	4	8	5	per
CR	*Cirsium vulgare*	forb	7	5	6	6	per
SC	*Clematis vitalba*	forb	6	4	8	5	per
SC/CSR	*Clinopodium vulgare*	forb	7	4	7	4	per
CR	*Convolvulus arvensis*	forb	7	4	8	6	per
R/SR	*Crepis capillaris*	forb	7	4	7	4	ann
SR	*Dactylorhiza fuchsii*	forb	7	8	7	3	per
C/CSR	*Galium mollugo*	forb	7	4	7	4	per
CSR	*Galium pumilum*	forb	7	4	8	3	per
SC/CSR	*Galium verum*	forb	7	4	6	2	per
R/SR	*Geranium columbinum*	forb	7	4	7	7	ann

continued over ...

... RainDrop species list continued

CSR class	species	plant group	EL	EM	ER	EN	life history
R/CR	*Geranium dissectum*	forb	7	5	7	6	ann
R/CSR	*Geranium molle*	forb	7	5	6	5	ann
CR/CSR	*Glechoma hederacea*	forb	6	6	7	7	per
C/CSR	*Heracleum sphondylium*	forb	7	5	7	7	per
SR	*Hyacinthoides sp*	forb	5	5	5	6	per
SC/CSR	*Hypericum hirsutum*	forb	6	5	7	5	per
CR/CSR	*Hypericum perforatum*	forb	7	4	7	5	per
CSR	*Hypochaeris radicata*	forb	8	4	5	3	per
CSR	*Knautia arvensis*	forb	7	3	8	4	per
R	*Lamium purpureum*	forb	6	5	7	7	ann
R/CSR	*Leontodon autumnalis*	forb	8	6	6	4	per
CSR	*Leontodon hispidus*	forb	8	4	7	3	per
SR	*Linum catharticum*	forb	8	5	7	2	bi
C/CSR	*Malva moschata*	forb	7	3	7	4	per
R/CR	*Odontites vernus*	forb	7	5	6	5	ann
CR	*Pastinaca sativa*	forb	7	4	7	5	bi
CSR	*Plantago lanceolata*	forb	7	5	6	4	per
CR/CSR	*Potentilla reptans*	forb	7	5	7	5	per
S/CSR	*Primula veris*	forb	7	4	7	3	per
CSR	*Prunella vulgaris*	forb	7	5	6	4	per
CSR	*Ranunculus acris*	forb	7	6	6	4	per
R	*Ranunculus parviflorus*	forb	7	5	6	5	per
CR	*Ranunculus repens*	forb	6	7	6	7	per
R/SR	*Rhinanthus minor*	forb	7	5	6	4	ann
CR/CSR	*Rumex crispus*	forb	8	6	7	6	per
S/CSR	*Sanguisorba minor*	forb	7	4	8	3	per
SC/CSR	*Senecio erucifolius*	forb	7	5	7	5	per
CR/CSR	*Senecio jacobaea*	forb	7	4	6	4	bi
R/SR	*Sherardia arvensis*	forb	7	4	6	4	ann
R/CR	*Sonchus asper*	forb	7	5	7	6	ann
R/CR	*Sonchus oleraceus*	forb	7	5	7	7	ann
R/CSR	*Taraxacum agg*	forb	7	5	7	6	per
S	*Thymus polytrichus*	forb	8	4	6	2	per
CR/CSR	*Tragopogon pratensis*	forb	8	4	7	5	per
CSR	*Veronica chamaedrys*	forb	6	5	6	5	per
S/CSR	*Viola hirta*	forb	7	4	8	2	per
SC/CSR	*Astragalus glycyphyllos*	legume	6	4	7	3	per
S/CSR	*Lotus corniculatus*	legume	7	4	6	2	per
R/CSR	*Medicago lupulina*	legume	7	4	8	4	per
R/SR	*Trifolium campestre*	legume	8	4	6	4	ann
CSR	*Trifolium pratense*	legume	7	5	7	5	per
CR/CSR	*Trifolium repens*	legume	7	5	6	6	per
C/CSR	*Vicia cracca*	legume	7	6	7	5	per
R/CR	*Vicia hirsuta*	legume	7	4	8	2	ann
SR/CSR	*Vicia sativa*	legume	7	4	7	4	ann
CSR	*Acer sp*	woody	5	5	7	6	per
SC	*Corylus avellana*	woody	4	5	6	6	per
SC	*Crataegus monogyna*	woody	6	5	7	6	per
C/SC	*Fraxinus excelsior*	woody	5	6	7	6	per
SC	*Rubus fruticosus*	woody	6	6	6	6	per

Appendix 2.3 RainDrop species abundance summaries

Species that were observed only in the autumn surveys (2016, 2017) are not included in this summary as they have not been included in analysis: *Centaurea scabiosa, Festuca arundinacea, Geranium molle, Hypochaeris radicata, Lamium purpureum, Phleum bertolonii*.

June 2016 (baseline) species abundance summaries																
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species / nitrogen treatment	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Acer sp.	0	0	0	0	0	0.002	0	0	0.002	0	0	0	0	0	0	0
Achillea millefolium	0	0	0	0	0.004	0	0	0	0	0	0	0	0	0	0	0
Agrimonia eupatoria	0.022	0.026	0.008	0.016	0.02	0.026	0.052	0.024	0.018	0.03	0.008	0.010	0.03	0.012	0.028	0.038
Agrostis capillaris	0	0.002	0	0	0	0	0	0	0	0.002	0.010	0.006	0	0	0	0
Agrostis stolonifera	0.01	0	0	0.004	0.004	0.004	0	0.006	0.004	0.006	0.008	0	0	0.014	0	0
Anacamptis pyramidalis	0	0	0.002	0.004	0	0.002	0.002	0.002	0.002	0.002	0	0	0.002	0.002	0	0
Anthoxanthum odoratum	0.004	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0
Anthriscus sylvestris	0	0	0	0	0	0	0	0	0	0	0	0	0	0.002	0.002	0.004
Arrhenatherum elatius	0.124	0.040	0.084	0.086	0.066	0.150	0.044	0.096	0.022	0.026	0.040	0.14	0.048	0.040	0.070	0.158
Astragalus glycyphyllos	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.002

continued over ....

	June 2016 (baseline) continued ...															
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species \ **nitrogen treatment**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Brachypodium pinnatum	0.028	0.080	0.130	0.036	0.110	0.040	0.040	0.124	0.076	0.024	0.016	0.008	0.014	0.026	0.016	0.004
Brachypodium sylvaticum	0.052	0.054	0.014	0.016	0.026	0.068	0.038	0.060	0.030	0.024	0.046	0.026	0.018	0.010	0.032	0.008
Bromopsis erecta	0.016	0.040	0.020	0.030	0.040	0	0.010	0.004	0.040	0.040	0.012	0.006	0.042	0.012	0.006	0.004
Bromus commutatus	0.002	0.018	0.014	0.046	0.070	0.022	0.100	0.010	0.036	0.010	0.006	0.006	0	0	0.030	0.010
Bromus hordeaceus	0.006	0.020	0.014	0	0	0.012	0.004	0.012	0	0.080	0.040	0.004	0.036	0.008	0.020	0
Bromus sterilis	0	0	0	0	0	0	0	0	0.012	0	0	0	0	0	0	0.010
Broomrape sp.	0	0.002	0	0	0	0.002	0	0	0	0	0	0	0.002	0	0.002	0.002
Carex caryophyllea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carex flacca	0.004	0.002	0	0.002	0	0	0.002	0	0.012	0.012	0.016	0.006	0	0	0	0
Carex sylvatica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centaurea nigra	0	0	0	0	0	0	0	0.008	0	0.024	0	0.024	0	0	0	0.004
Cerastium fontanum	0.002	0.002	0.004	0.006	0.006	0.008	0.004	0.002	0.004	0.004	0.006	0.004	0.01	0.006	0.006	0
Cerastium glomeratum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cirsium eriophorum	0.02	0.042	0.012	0.024	0.008	0.042	0.014	0.006	0	0.004	0	0.004	0.068	0.004	0.054	0.016
Cirsium vulgare	0	0	0.008	0	0	0	0	0	0	0	0.002	0	0	0	0	0
Clematis vitalba	0.016	0	0.002	0	0	0	0	0	0	0.100	0	0.06	0.004	0	0	0

continued over ....

	June 2016 (baseline) continued...															
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species \ **nitrogen treatment**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Clinopodium vulgare	0.062	0.132	0.144	0.110	0.064	0.074	0.096	0.070	0.148	0.076	0.148	0.086	0.120	0.08	0.168	0.198
Convolvulus arvensis	0.002	0.002	0	0.002	0	0.002	0	0.002	0.002	0.002	0	0	0	0.002	0	0
Corylus avellana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crataegus monogyna	0.014	0.004	0.002	0.002	0.006	0.006	0.008	0	0.002	0.004	0.006	0.006	0.010	0.002	0.002	0.004
Crepis capillaris	0.016	0.008	0.004	0.010	0.010	0.022	0.010	0.024	0.016	0.008	0.010	0.014	0.016	0.018	0.020	0.008
Cynosurus cristatus	0	0	0.002	0.022	0	0	0	0	0	0	0	0.010	0.006	0	0	0.002
Dactylis glomerata	0.014	0.006	0.014	0.016	0.084	0.024	0.072	0.046	0.032	0.034	0.038	0.044	0.046	0.072	0.036	0.056
Dactylorhiza fuchsii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Festuca ovina	0	0.024	0	0	0	0	0	0.024	0	0	0	0	0	0	0	0
Festuca rubra	0.080	0.022	0.064	0.048	0.050	0.094	0.072	0.064	0.064	0.042	0.078	0.086	0.108	0.034	0.020	0.092
Fraxinus excelsior	0	0	0.002	0	0.002	0.002	0	0.002	0	0.002	0.002	0.002	0.004	0	0	0
Galium mollugo	0	0.024	0	0.006	0.020	0.050	0.002	0.002	0	0	0	0	0	0.008	0.004	0.004
Galium pumilum	0	0	0	0	0	0	0	0.002	0.002	0	0.002	0	0	0	0	0
Galium verum	0.004	0	0.002	0	0.030	0.040	0.002	0.004	0.002	0.072	0.010	0	0.030	0.086	0.032	0.008
Geranium columbinum	0.004	0.006	0.014	0.006	0.008	0.012	0.010	0.004	0.002	0.002	0.004	0.004	0.010	0.006	0.014	0.012

continued over ....

	June 2016 (baseline) continued ...															
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species \ **nitrogen treatment**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Geranium dissectum	0.016	0.016	0.012	0.006	0.012	0.010	0.010	0.008	0.012	0.002	0.014	0.008	0.018	0.022	0.024	0.024
Glechoma hederacea	0.004	0.002	0.002	0.004	0	0	0	0	0	0.002	0	0	0	0	0.004	0.002
Helictotrichon pratense	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helictotrichon pubescens	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0	0
Heracleum sphondylium	0	0	0	0	0.02	0.006	0	0	0	0	0	0	0	0	0.004	0
Hieracium sp	0.008	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Holcus lanatus	0.048	0.012	0.064	0.062	0.042	0.052	0.022	0.048	0.022	0.036	0.02	0.026	0.024	0.012	0.082	0.022
Hyacinthoides non-scripta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypericum hirsutum	0.006	0.008	0.008	0.002	0.004	0.004	0.008	0.006	0.002	0	0.004	0.004	0.004	0.004	0.006	0.004
Hypericum perforatum	0.008	0.002	0.004	0	0.008	0.008	0.012	0.006	0.006	0.006	0.008	0.004	0.002	0.010	0.004	0.01
Knautia arvensis	0	0.008	0	0	0	0	0.002	0.002	0	0.002	0	0.002	0.008	0	0	0.004
Leontodon autumnalis	0	0	0	0	0	0	0	0	0.002	0.002	0.002	0.002	0	0	0	0
Leontodon hispidus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Linum catharticum	0.002	0.004	0.002	0.004	0.002	0.002	0.002	0.002	0.004	0.004	0.006	0.004	0.002	0.002	0.002	0.002
Lolium multiflorum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lolium perenne	0.050	0.010	0	0.040	0	0.060	0.080	0.010	0	0.026	0.050	0.048	0.034	0.022	0.010	0

continued over ....

	June 2016 (baseline) continued ...															
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species \ **nitrogen treatment**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Lotus corniculatus	0.102	0.064	0.140	0.084	0.080	0.050	0.050	0.050	0.198	0.100	0.120	0.122	0	0	0.010	0
Luzula campestris	0	0	0	0	0	0	0	0	0.002	0	0	0.002	0	0	0	0
Malva moschata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medicago lupulina	0.028	0.046	0.032	0.036	0.016	0.026	0.006	0.022	0.076	0.036	0.018	0.078	0.014	0.020	0.028	0.036
Odontites vernus	0.002	0.002	0.006	0.002	0.002	0.004	0.002	0	0.004	0	0.002	0	0.002	0.002	0.006	0.002
Pastinaca sativa	0.044	0.046	0.028	0.03	0.018	0.044	0.048	0.040	0.030	0.026	0.036	0.018	0.086	0.028	0.040	0.034
Plantago lanceolata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poa annua	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0
Poa pratensis	0.020	0.014	0.030	0.010	0	0.012	0.006	0.008	0.020	0.040	0.022	0.020	0.008	0	0.020	0.040
Poa trivialis	0.026	0.030	0.006	0.032	0.056	0.072	0.070	0.032	0.032	0.022	0.070	0.020	0.096	0.110	0.038	0.028
Potentilla reptans	0.018	0.024	0.024	0.020	0.030	0.018	0.024	0.016	0.016	0.006	0.022	0.018	0.022	0.064	0.024	0.016
Primula veris	0	0	0.002	0	0	0	0	0.002	0	0	0	0.002	0	0	0	0
Prunella vulgaris	0.006	0.008	0.010	0.010	0.006	0.006	0.006	0.008	0.010	0.006	0.006	0.006	0.012	0.020	0.018	0.012
Prunus sp	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0	0
Quercus sp	0	0.002	0	0	0	0	0	0.002	0.002	0	0	0	0	0	0.002	0
Ranunculus acris	0.008	0.004	0.008	0.010	0	0.006	0.002	0.002	0	0.004	0.004	0.006	0	0.006	0	0.004

continued over ....

	June 2016 (baseline) continued ...															
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species \ **nitrogen treatment**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Ranunculus parviflorus	0	0	0	0.002	0	0	0	0	0	0	0	0.002	0	0	0	0
Ranunculus repens	0	0	0	0.004	0.002	0	0.008	0	0.006	0	0.002	0.004	0	0.004	0.002	0.004
Rhinanthus minor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rosa sp.	0.008	0	0.002	0.002	0.008	0.002	0	0.004	0	0.002	0	0	0	0	0	0
Rubus fruticosus	0.002	0.002	0	0.004	0.014	0.008	0.014	0.008	0	0	0	0	0.008	0	0	0.004
Rumex crispus	0	0	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0
Sanguisorba minor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Senecio erucifolius	0.004	0.002	0.002	0.002	0.002	0	0.004	0	0.004	0	0.002	0.004	0.004	0.002	0	0
Senecio jacobaea	0	0	0	0.004	0.002	0	0.006	0.002	0	0	0	0.002	0	0.002	0	0
Sherardia arvensis	0.004	0	0	0	0.002	0	0	0	0.004	0	0	0	0	0	0	0
Sonchus asper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sonchus oleraceus	0	0.002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Taraxacum agg.	0.006	0.006	0.002	0.004	0.006	0.004	0	0	0	0	0.002	0	0	0	0.002	0.006
Thymus polytrichus	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0
Tragopogon pratensis	0	0	0	0	0	0.002	0	0	0	0.002	0.002	0	0	0	0	0
Trifolium campestre	0.002	0.006	0	0.004	0.002	0	0	0	0	0	0	0	0	0	0	0

continued over ....

	June 2016 (baseline) continued ...															
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species \ **nitrogen treatment**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Trifolium pratense	0.006	0.028	0.016	0.018	0.006	0.004	0.010	0.010	0.008	0.006	0.028	0.018	0.002	0.010	0	0.050
Trifolium repens	0.104	0.040	0.050	0.088	0.034	0.022	0.030	0.032	0.036	0.032	0.060	0.036	0.046	0.082	0.062	0.118
Trisetum flavescens	0.214	0.344	0.242	0.324	0.174	0.160	0.260	0.304	0.234	0.350	0.270	0.244	0.280	0.286	0.320	0.230
Veronica chamaedrys	0.030	0.014	0.012	0.028	0.008	0.014	0.012	0.032	0.016	0.036	0.014	0.020	0.026	0.032	0.012	0.032
Vicia cracca	0	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0
Vicia hirsuta	0	0	0	0	0	0.002	0	0.002	0	0	0	0	0	0	0	0.002
Vicia sativa	0.010	0.008	0.010	0.010	0.016	0.006	0.014	0.008	0.014	0.014	0.016	0.012	0.006	0.014	0.006	0.020
Viola hirta	0.002	0.006	0.002	0.002	0	0	0	0	0	0.002	0	0	0.002	0.002	0.002	0.002
Viola reichenbachiana	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0

	June 2017 species abundance summaries															
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species \ nitrogen treatment	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Acer sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achillea millefolium	0	0	0	0	0.012	0	0	0	0	0	0	0	0	0	0	0
Agrimonia eupatoria	0.020	0.022	0.024	0.006	0.020	0.012	0.048	0.022	0.006	0.018	0.014	0.014	0.018	0.018	0.008	0.046
Agrostis capillaris	0	0.004	0.006	0.002	0.004	0.002	0	0	0	0.002	0.004	0.002	0.006	0	0.006	0.012
Agrostis stolonifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anacamptis pyramidalis	0.002	0.002	0.002	0.004	0.004	0.004	0.004	0.004	0.002	0.002	0.004	0.004	0.002	0.006	0.006	0.002
Anthoxanthum odoratum	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0	0
Anthriscus sylvestris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arrhenatherum elatius	0.212	0.136	0.122	0.084	0.146	0.054	0.158	0.182	0.130	0.130	0.154	0.082	0.082	0.074	0.048	0.094
Astragalus glycyphyllos	0	0	0	0	0	0	0	0	0	0	0	0.024	0	0.008	0	0
Brachypodium pinnatum	0.096	0.064	0.192	0.050	0.200	0.094	0.150	0.240	0.078	0.056	0.106	0.056	0.022	0.050	0.040	0.056
Brachypodium sylvaticum	0.026	0.026	0.018	0.016	0.012	0.138	0.026	0.028	0.002	0.004	0.012	0.018	0.004	0.006	0.006	0.002
Bromopsis erecta	0.014	0.070	0.042	0.010	0.030	0.006	0.020	0.008	0.002	0	0.036	0.022	0.054	0.010	0.024	0.014
Bromus commutatus	0.004	0.010	0.004	0.004	0	0	0.006	0	0	0.012	0.012	0.002	0	0	0.006	0
Bromus hordeaceus	0.002	0	0	0.008	0.004	0.014	0.024	0.014	0.008	0	0.002	0.002	0.008	0.020	0.006	0.012
Bromus sterilis	0	0	0.004	0	0	0.008	0.006	0.002	0.002	0	0	0	0.008	0	0	0
Broomrape sp.	0	0.004	0	0.004	0	0.002	0.002	0.002	0	0	0.002	0	0	0	0	0
Carex caryophyllea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carex flacca	0.004	0	0	0	0.002	0	0.002	0	0	0	0.002	0.002	0	0	0	0
Carex sylvatica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centaurea nigra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

continued over ....

	June 2017 continued ...															
rainfall treatment	ambient17				irrigated				procedural control				drought			
species \ nitrogen treatment	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water
Cerastium fontanum	0	0	0.002	0	0	0	0.004	0.002	0	0	0.002	0	0	0.002	0.002	0.002
Cerastium glomeratum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cirsium eriophorum	0.008	0.052	0.004	0	0.002	0.002	0	0.022	0.002	0.004	0	0.004	0.012	0	0.004	0.004
Cirsium vulgare	0	0	0.004	0.002	0	0	0	0	0	0	0.002	0	0	0	0	0
Clematis vitalba	0.024	0	0.002	0	0	0	0	0	0	0.100	0	0.120	0	0	0	0
Clinopodium vulgare	0.08	0.078	0.032	0.050	0.048	0.034	0.050	0.040	0.038	0.048	0.064	0.040	0.048	0.024	0.048	0.034
Convolvulus arvensis	0	0	0	0	0.002	0.002	0.002	0.002	0	0	0	0	0	0.002	0	0.002
Corylus avellana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crataegus monogyna	0.032	0.004	0.006	0.004	0.006	0.012	0.024	0	0.004	0.006	0.012	0.010	0.020	0.008	0.004	0.008
Crepis capillaris	0.008	0.014	0.004	0.022	0.018	0.022	0.018	0.024	0.018	0.018	0.018	0.030	0.016	0.012	0.022	0.012
Cynosurus cristatus	0.004	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.002
Dactylis glomerata	0.008	0.016	0.008	0.020	0.022	0.018	0.030	0.028	0.006	0.008	0.010	0.010	0.018	0.034	0.020	0.032
Dactylorhiza fuchsii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Festuca ovina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Festuca rubra	0.122	0.006	0.028	0.022	0.008	0.028	0.018	0.034	0.008	0.006	0.008	0.012	0.004	0.010	0.016	0.020
Fraxinus excelsior	0.004	0.002	0.002	0	0.002	0.002	0	0.002	0.002	0.004	0.002	0.002	0.004	0	0.004	0
Galium mollugo	0	0.050	0	0.040	0.008	0.050	0.006	0.002	0	0	0	0	0	0.010	0.012	0.008
Galium pumilum	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0
Galium verum	0.006	0.004	0.004	0	0.032	0.006	0	0.024	0.004	0.160	0.052	0.006	0.024	0.104	0.098	0.032
Geranium columbinum	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.008	0.002	0.004	0.004	0.004	0.002	0.002	0.002	0.002
Geranium dissectum	0	0.002	0.002	0.004	0.006	0.002	0.006	0.002	0.002	0.002	0.006	0.002	0.002	0.006	0.004	0.008

continued over ....

	June 2017 continued ...															
rainfall treatment	ambient				irrigated				procedural control				drought			
species \ nitrogen treatment	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water
Glechoma hederacea	0.002	0	0	0.002	0	0	0	0.002	0	0	0	0	0	0.002	0.002	0
Helictotrichon pratense	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helictotrichon pubescens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heracleum sphondylium	0.002	0.014	0	0.002	0	0	0.006	0	0	0	0	0	0	0	0	0
Hieracium sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Holcus lanatus	0.046	0.028	0.030	0.048	0.046	0.058	0.044	0.042	0.014	0.020	0.020	0.016	0.034	0.014	0.012	0.014
Hyacinthoides non-scripta	0	0	0	0	0	0	0	0	0	0	0	0.002	0.002	0	0	0
Hypericum hirsutum	0.002	0.004	0.008	0	0.006	0.004	0.002	0.002	0	0.002	0.002	0.002	0.002	0.004	0.004	0.002
Hypericum perforatum	0.004	0.002	0.008	0	0.022	0.022	0.004	0.014	0.010	0.014	0.002	0.004	0.012	0.006	0.008	0.010
Knautia arvensis	0	0	0	0	0	0.004	0.006	0.024	0	0.002	0.006	0	0.010	0	0	0.012
Leontodon autumnalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leontodon hispidus	0	0	0	0.002	0	0	0	0	0	0	0	0.002	0.004	0	0	0
Linum catharticum	0.006	0.008	0.008	0.006	0.006	0.008	0.008	0.004	0.010	0.006	0.006	0.010	0.006	0.006	0.006	0.006
Lolium multiflorum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.004	0
Lolium perenne	0.010	0.004	0	0.032	0.020	0.032	0.044	0.026	0	0	0.010	0.006	0.008	0.020	0.014	0.016
Lotus corniculatus	0.154	0.080	0.146	0.070	0.120	0.132	0.090	0.144	0.270	0.154	0.198	0.298	0.006	0.012	0.026	0.010
Luzula campestris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malva moschata	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0
Medicago lupulina	0.008	0.030	0.010	0.022	0.016	0.004	0.008	0.028	0.018	0.020	0.020	0.036	0	0.008	0.002	0.002
Odontites vernus	0.002	0.004	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0
Pastinaca sativa	0.030	0.014	0.038	0.018	0.022	0.032	0.024	0.028	0.04	0.022	0.032	0.034	0.03	0.018	0.038	0.038

continued over ....

	June 2017 continued ...															
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species / nitrogen treatment	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Plantago lanceolata	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0	0
Poa annua	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poa pratensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poa trivialis	0.004	0	0.002	0	0.006	0.006	0.002	0	0	0	0	0.002	0.012	0.002	0	0
Potentilla reptans	0.082	0.130	0.088	0.146	0.058	0.036	0.064	0.024	0.018	0.034	0.034	0.022	0.052	0.038	0.042	0.060
Primula veris	0	0	0.002	0	0	0.002	0	0.002	0	0	0	0.002	0.004	0	0	0
Prunella vulgaris	0.010	0.012	0.006	0.008	0.006	0.028	0.014	0.016	0.018	0.008	0.006	0.018	0.016	0.006	0.012	0.006
Prunus sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0.002	0.004	0
Quercus sp	0	0	0	0.002	0	0	0	0	0	0	0	0.002	0	0	0	0
Ranunculus acris	0	0.004	0	0.004	0.002	0.002	0.002	0	0.002	0.002	0	0.002	0	0	0	0
Ranunculus parviflorus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus repens	0.002	0	0.002	0	0	0.004	0.004	0.002	0.002	0	0	0	0	0.002	0	0
Rhinanthus minor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rosa sp.	0.002	0	0	0.002	0.004	0.004	0	0	0	0	0	0	0	0	0	0
Rubus fruticosus	0.012	0.002	0	0.002	0.012	0.008	0.018	0.010	0	0	0	0	0.004	0	0	0.008
Rumex crispus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sanguisorba minor	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0.002	0
Senecio erucifolius	0	0	0.004	0.010	0.002	0	0.004	0.002	0	0	0.002	0.004	0.002	0.002	0.002	0.002
Senecio jacobaea	0	0	0	0	0.002	0	0.002	0	0	0	0.002	0	0.002	0.002	0	0
Sherardia arvensis	0	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0
Sonchus asper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

continued over ....

	June 2017 continued ...															
rainfall treatment	ambient				irrigated				procedural control				drought			
species \ nitrogen treatment	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water
Sonchus oleraceus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Taraxacum agg.	0.002	0	0	0	0	0.002	0	0.004	0	0	0	0.002	0	0	0	0
Thymus polytrichus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tragopogon pratensis	0.002	0.002	0	0.002	0.002	0.004	0	0	0	0.002	0	0	0.002	0	0.002	0
Trifolium campestre	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium pratense	0.012	0.116	0.038	0.074	0.022	0.042	0.066	0.024	0.006	0.002	0.040	0.024	0.004	0.022	0.010	0.068
Trifolium repens	0.088	0.082	0.132	0.076	0.054	0.066	0.094	0.118	0.050	0.076	0.098	0.060	0.070	0.028	0.016	0.092
Trisetum flavescens	0.010	0.005	0.020	0.048	0.048	0.044	0.048	0.024	0.078	0.016	0.034	0.040	0.030	0.024	0.046	0.022
Veronica chamaedrys	0.010	0.006	0.006	0.006	0.010	0.008	0.008	0.006	0.004	0.010	0.006	0.008	0.004	0.006	0.008	0.006
Vicia cracca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vicia hirsuta	0	0.002	0.002	0.006	0	0	0	0.002	0	0.002	0.002	0	0	0	0	0.002
Vicia sativa	0	0.004	0.002	0.002	0.006	0.002	0.004	0.008	0	0	0.004	0.002	0.002	0.002	0	0
Viola hirta	0	0.004	0.004	0.002	0	0.002	0	0	0	0	0	0	0.002	0	0	0
Viola reichenbachiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

June 2018 species abundance summaries																
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species \ nitrogen treatment	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Acer sp.	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0
Achillea millefolium	0	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0
Agrimonia eupatoria	0.006	0.016	0.018	0.010	0.012	0.020	0.030	0.018	0.010	0.012	0.008	0.016	0.020	0.042	0.020	0.050
Agrostis capillaris	0.002	0.002	0.004	0.004	0	0.004	0	0.002	0	0	0.006	0.002	0.008	0.002	0.002	0.008
Agrostis stolonifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anacamptis pyramidalis	0	0.004	0.004	0	0.004	0.004	0.002	0.004	0.002	0.002	0.002	0.004	0.004	0.004	0.004	0.004
Anthoxanthum odoratum	0	0	0.004	0	0.004	0	0	0	0.004	0	0	0	0	0	0.004	0
Anthriscus sylvestris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arrhenatherum elatius	0.112	0.166	0.106	0.134	0.09	0.154	0.100	0.098	0.140	0.150	0.156	0.132	0.076	0.112	0.074	0.076
Astragalus glycyphyllos	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachypodium pinnatum	0.146	0.070	0.112	0.022	0.170	0.074	0.168	0.118	0.074	0.040	0.058	0.020	0.070	0.084	0.152	0.118
Brachypodium sylvaticum	0.008	0.018	0.004	0.004	0.020	0.036	0.008	0.016	0.004	0.010	0.014	0.012	0.010	0.020	0.018	0.010
Bromopsis erecta	0.020	0.042	0.078	0.076	0.028	0.030	0.028	0.048	0.006	0	0.038	0.018	0.030	0.020	0.008	0.048
Bromus commutatus	0.002	0.016	0.002	0.008	0	0	0	0	0.002	0.002	0.006	0.002	0.006	0.006	0.006	0.004
Bromus hordeaceus	0	0	0.002	0	0	0.002	0.004	0	0.002	0	0	0	0.004	0	0.002	0
Bromus sterilis	0	0.002	0.006	0.002	0	0.002	0	0	0.002	0	0	0	0	0	0.002	0.008
Broomrape sp.	0.002	0	0	0.004	0	0.004	0.002	0.002	0.002	0	0	0.002	0.002	0	0	0
Carex caryophyllea	0	0.002	0.002	0	0	0	0.002	0	0	0	0.002	0	0	0	0	0
Carex flacca	0.002	0.004	0.004	0.002	0.002	0	0.004	0	0.004	0	0.004	0.008	0.002	0	0.002	0.002
Carex sylvatica	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0	0	0
Centaurea nigra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

continued over ....

	June 2018 continued ...															
rainfall treatment	ambient				irrigated				procedural control				drought			
species \ nitrogen treatment	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water
Cerastium fontanum	0.004	0.002	0.006	0.006	0.006	0.004	0.006	0.004	0.008	0	0.004	0	0.006	0.004	0.002	0.002
Cerastium glomeratum	0	0	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0
Cirsium eriophorum	0.006	0	0.004	0.010	0	0.012	0.004	0.004	0.006	0.020	0.008	0.012	0.062	0.006	0	0.014
Cirsium vulgare	0	0	0	0.004	0	0	0	0	0	0	0	0	0	0	0	0
Clematis vitalba	0.030	0	0	0	0	0	0	0	0	0.120	0	0.110	0	0	0	0
Clinopodium vulgare	0.022	0.024	0.024	0.024	0.016	0.038	0.010	0.010	0.064	0.042	0.020	0.014	0.064	0.070	0.058	0.040
Convolvulus arvensis	0.002	0.002	0.006	0.004	0	0.004	0	0	0	0.004	0.002	0.004	0	0.004	0.002	0.004
Corylus avellana	0	0	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0
Crataegus monogyna	0.012	0.002	0	0.002	0.008	0.006	0.002	0	0	0.004	0.004	0.010	0.004	0.014	0.002	0.012
Crepis capillaris	0.008	0.008	0.008	0.008	0.014	0.010	0.004	0.004	0.012	0.012	0.02	0.008	0.004	0.024	0.006	0.014
Cynosurus cristatus	0	0.004	0.006	0.014	0.002	0	0	0	0.002	0.004	0.002	0.004	0.002	0	0.004	0.004
Dactylis glomerata	0.006	0.018	0.012	0.016	0.030	0.020	0.034	0.018	0.004	0.020	0.010	0.018	0.026	0.020	0.022	0.002
Dactylorhiza fuchsii	0	0	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0
Festuca ovina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Festuca rubra	0.070	0.018	0.026	0.026	0.010	0.016	0.012	0.020	0.008	0.012	0.014	0.012	0.004	0.010	0.008	0.006
Fraxinus excelsior	0.002	0	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0
Galium mollugo	0	0.014	0	0.002	0.008	0.026	0.002	0.002	0	0	0	0.006	0.002	0.002	0.014	0.006
Galium pumilum	0.002	0	0	0.010	0	0	0	0	0.002	0	0.002	0	0	0	0	0
Galium verum	0.006	0.004	0.006	0.002	0.008	0.004	0	0.016	0.002	0.036	0.008	0.006	0.012	0.056	0.042	0.010
Geranium columbinum	0.008	0.008	0.008	0.008	0.008	0.008	0.004	0.006	0.006	0.006	0.006	0.004	0.010	0.010	0.006	0.010
Geranium dissectum	0.004	0.002	0.006	0	0.006	0.002	0.004	0.002	0.002	0.004	0.004	0	0.006	0.006	0.008	0.004

continued over ....

	June 2018 continued ...															
rainfall treatment	ambient				irrigated				procedural control				drought			
species \ nitrogen treatment	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water
Glechoma hederacea	0.006	0	0.002	0.002	0	0.004	0.002	0	0	0.002	0.004	0	0.008	0.004	0.008	0.004
Helictotrichon pratense	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0	0	0
Helictotrichon pubescens	0.004	0.002	0.004	0.004	0	0	0.006	0	0	0	0	0	0.002	0.010	0	0
Heracleum sphondylium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hieracium sp	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0.002	0	0
Holcus lanatus	0.050	0.044	0.056	0.064	0.036	0.042	0.046	0.046	0.022	0.014	0.026	0.022	0.026	0.026	0.030	0.026
Hyacinthoides non-scripta	0	0	0	0.002	0	0	0	0.002	0	0	0	0.002	0.004	0	0	0
Hypericum hirsutum	0.002	0.006	0.006	0.004	0	0.002	0.002	0	0.002	0.004	0.010	0.006	0.006	0.008	0.002	0.002
Hypericum perforatum	0.008	0.004	0.006	0.002	0.012	0.006	0.006	0.008	0.006	0.016	0	0.004	0.008	0.008	0.004	0.014
Knautia arvensis	0	0.002	0	0	0	0	0.010	0.012	0	0	0	0.042	0.008	0	0	0.010
Leontodon autumnalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0.002	0	0
Leontodon hispidus	0.004	0	0	0.004	0	0	0	0	0	0	0	0	0.002	0.004	0	0.002
Linum catharticum	0.004	0.004	0.006	0.006	0.004	0.006	0.006	0.004	0.010	0.004	0.008	0.008	0.004	0.008	0.004	0.006
Lolium multiflorum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lolium perenne	0.014	0.012	0.002	0.006	0.020	0.04	0.008	0.014	0.010	0.004	0	0.004	0.006	0.016	0.014	0.006
Lotus corniculatus	0.190	0.110	0.160	0.034	0.0800	0.078	0.088	0.114	0.294	0.202	0.240	0.274	0.032	0.016	0.106	0.022
Luzula campestris	0	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0
Malva moschata	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0
Medicago lupulina	0.042	0.098	0.032	0.040	0.102	0.148	0.126	0.084	0.104	0.054	0.060	0.082	0.052	0.096	0.084	0.108
Odontites vernus	0	0	0	0	0	0	0.002	0	0	0	0	0	0	0	0	0.002
Pastinaca sativa	0.022	0.002	0.020	0.060	0.020	0.018	0.014	0.034	0.052	0.048	0.030	0.040	0.032	0.048	0.036	0.032

continued over ....

	June 2018 continued ...															
rainfall treatment	**ambient**				**irrigated**				**procedural control**				**drought**			
species / nitrogen treatment	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**	**none**	**Nox**	**Nred**	**water**
Plantago lanceolata	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0	0	0
Poa annua	0	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0
Poa pratensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poa trivialis	0	0	0.002	0.008	0.004	0.004	0.004	0	0.008	0.012	0.002	0.006	0.004	0.006	0	0.004
Potentilla reptans	0.020	0.022	0.022	0.016	0.01	0.038	0.044	0.020	0.014	0.006	0.02	0.006	0.028	0.044	0.028	0.058
Primula veris	0	0	0.002	0	0.002	0	0.002	0.002	0.002	0	0.002	0.002	0	0	0	0.002
Prunella vulgaris	0.014	0.008	0.016	0.010	0.010	0.022	0.018	0.022	0.036	0.026	0.016	0.012	0.024	0.02	0.022	0.026
Prunus sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus acris	0	0	0	0	0.002	0.004	0.002	0	0	0.002	0	0	0	0	0	0
Ranunculus parviflorus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus repens	0.004	0.004	0.004	0.004	0	0.002	0.004	0.002	0.006	0.004	0.002	0.006	0	0.002	0.002	0.002
Rhinanthus minor	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0	0
Rosa sp.	0.002	0	0.002	0.002	0	0.002	0	0	0	0	0	0	0	0	0	0
Rubus fruticosus	0.004	0	0	0.002	0	0.002	0.002	0.002	0	0	0	0	0.002	0	0	0.004
Rumex crispus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sanguisorba minor	0	0	0	0	0	0	0	0	0.004	0	0	0	0	0	0.002	0
Senecio erucifolius	0.002	0	0.004	0.008	0.002	0	0.004	0.002	0	0.002	0.002	0.014	0.018	0.006	0.006	0
Senecio jacobaea	0	0	0	0	0	0	0.002	0	0	0	0.004	0	0	0	0	0
Sherardia arvensis	0	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0
Sonchus asper	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0

continued over ....

	June 2018 continued ...															
rainfall treatment	ambient				irrigated				procedural control				drought			
species \ nitrogen treatment	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water	none	Nox	Nred	water
Sonchus oleraceus	0	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0
Taraxacum agg.	0.004	0.004	0.002	0.006	0.002	0.002	0.006	0.002	0.002	0.002	0.004	0.006	0.010	0.004	0.008	0.006
Thymus polytrichus	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0
Tragopogon pratensis	0.002	0.002	0	0.002	0.004	0.002	0.004	0	0.004	0.004	0.002	0	0.002	0.002	0.002	0.002
Trifolium campestre	0	0	0	0	0.002	0	0	0	0	0	0	0	0.004	0	0	0
Trifolium pratense	0.044	0.070	0.036	0.090	0.012	0.016	0.062	0.016	0.022	0.016	0.050	0.042	0.010	0.022	0.014	0.090
Trifolium repens	0.174	0.212	0.168	0.166	0.208	0.178	0.290	0.298	0.080	0.14	0.124	0.114	0.050	0.070	0.028	0.066
Trisetum flavescens	0.044	0.054	0.046	0.036	0.068	0.018	0.056	0.040	0.050	0.028	0.038	0.038	0.048	0.028	0.038	0.028
Veronica chamaedrys	0.010	0.012	0.008	0.008	0.006	0.010	0.012	0.008	0.010	0.010	0.010	0.006	0.010	0.010	0.012	0.012
Vicia cracca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vicia hirsuta	0	0	0	0	0	0	0	0	0.002	0	0	0.004	0	0	0	0.002
Vicia sativa	0.010	0.010	0.010	0.008	0.010	0.008	0.010	0.010	0.008	0.010	0.008	0.008	0.010	0.008	0.010	0.008
Viola hirta	0.002	0	0.004	0.004	0	0.002	0.002	0	0	0.004	0.002	0	0.004	0	0.002	0.004
Viola reichenbachiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 3 Grassland surveys species information and abbreviated species names

Species' frequency – species were recorded as presence/absence data, so frequency is equal to the number of quadrats. For reference where species names have been abbreviated, e.g. in ordination plots.

Species observed within survey quadrats across the eleven sites. Number of quadrats and number of sites a species was observed in are given.

Key

Plant group: g = grasses and sedges; f = non-leguminous forbs; l = legumes; w = woody

Life history: per = perennial; a = annual; bi = biennial

Species	abbreviation	Plant group	Life history	No. of quadrats	No. of sites
Achillea millefolium	Achll_ml	f	per	3	1
Agrimonia eupatoria	Agrmn_pt	f	per	2	1
Agrostis capillaris	Agrsts_c	g	per	20	5
Anagallis arvensis	Anglls_r	f	annual	1	1
Anthoxanthum odoratum	Anthxnt_	g	per	103	10
Anthyllis vulneraria	Anthyll_	l	per	12	4
Aphanes arvensis	Aphns_rv	f	annual	4	1
Arabis hirsuta	Arbs_hrs	f	bi	1	1
Arrhenatherum elatius	Arrhnth_	g	per	22	6
Astragalus danicus	Astrgls_	l	per	1	1
Avenula pratensis	Avnl_prt	g	per	50	8
Bellis perennis	Blls_prn	f	per	2	1
Blackstonia perfoliata	Blckstn_	f	annual	2	1
Brachypodium pinnatum	Brchypdm_p	g	per	19	4
Brachypodium sylvaticum	Brchypdm_s	g	per	42	8
Briza media	Briza_md	g	per	65	9

continued over...

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Species	abbreviation	Plant group	Life history	No. of quadrats	No. of sites
Bromus commutatus	Brms_cmm	g	annual	10	2
Bromus erecta	Brms_rct	g	per	87	7
Bromus hordaceus	Brms_hrd	g	annual	7	2
Bromus sterilis	Brms_str	g	annual	1	1
Calluna vulgaris	Clln_vlg	w	per	1	1
Campanula rotundifolia	Cmpnl_rt	f	per	26	7
Carex caryophyllea	Crx_cryp	g	per	13	4
Carex flacca	Crx_flcc	g	per	146	10
Carex nigra	Carx_ngr	g	per	43	8
Carex pilulifera	Crx_pllf	g	per	1	1
Carlina vulgaris	Crln_vlg	f	per	2	2
Centaurea nigra	Cntr_ngr	f	per	21	5
Cerastium fontanum	Crstm_fn	f	per	6	2
Cirsium acaulon	Crsm_cln	f	per	9	3
Cirsium arvense	Crsm_rvn	f	per	2	2
Crataegus sp	Crtgs_sp	w	per	9	5
Crepis biennis	Crps_bnn	f	bi	23	3
Crepis capillaris	Crps_cpl	f	annual	4	2
Cynosurus cristatus	Cynsrs_c	g	per	19	6
Dactylis glomerata	Dctyls_g	g	per	17	7
Daucus carota	Dacs_crt	f	bi	3	2
Euphrasia sp	Ephrs_sp	f	annual	5	3
Festuca ovina	Festc_vn	g	per	103	11
Festuca rubra	Fstc_rbr	g	per	44	8
Filipendula vulgaris	Flpndl_v	f	per	26	4
Galium mollugo	Glm_mllg	f	per	2	1
Galium pumilum	Glm_pmlm	f	per	3	1
Galium sterneri	Glm_strn	f	per	2	2
Galium verum	Galm_vrm	f	per	30	8
Geranium columbinum	Grnm_clm	f	annual	1	1
Geranium pusillum	Grnm_psl	f	annual	3	1
Geranium sanguineum	Grnm_sng	f	per	1	1
Helianthemum nummularium	Hlnthmm_	f	per	92	8

continued over ...

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Species	abbreviation	Plant group	Life history	No. of quadrats	No. of sites
Helictotrichon pubescens	Hlcttrc_	g	per	13	5
Hieracium pilosella	Hrcm_pls	f	per	70	10
Hippocrepis comosa	Hppcrps_	l	per	6	3
Holcus lanatus	Hlcs_lnt	g	per	12	7
Hypericum hirsutum	Hyprcm_h	f	per	2	2
Hypericum montanum	Hyprcm_m	f	per	1	1
Hypericum perforatum	Hyprcm_p	f	per	5	2
Hypericum tetrapterum	Hyprcm_t	f	per	1	1
Hypochaeris radicata	Hypchrs_	f	per	21	2
Knautia arvensis	Knt_rvns	f	per	17	3
Koeleria macrantha	Klr_mcrn	g	per	32	8
Leontodon hispidus	Lntdn_hs	f	per	17	3
Leucanthemum vulgare	Lcnthmm_	f	per	4	2
Linum catharticum	Lnm_cthr	f	bi	59	8
Listera ovata	Listr_vt	f	per	2	2
Lithospermum officinale	Lthsprm_	f	per	1	1
Lotus corniculatus	Lts_crnc	l	per	149	11
Luzula campestris	Lzl_cmps	f	per	44	7
Luzula multiflora	Lzl_mltf	g	per	1	1
Medicago lupulina	Mdcg_lpl	l	annual	16	3
Nardus stricta	Nrds_str	g	per	1	1
Orchis mascula	Orchs_ms	f	per	10	5
Plantago lanceolata	Plntg_ln	f	per	105	11
Plantago media	Plntg_md	f	per	11	4
Poa pratensis	P_prtnss	g	per	1	1
Polygala vulgaris	Plygl_vl	f	per	32	9
Potentilla erecta	Ptntll_r	f	per	30	6
Potentilla sterilis	Ptntll_s	f	per	3	2
Primula veris	Prml_vrs	f	per	14	6
Prunella vulgaris	Prnll_vl	f	per	21	6
Ranunculus acris	Rnncls_c	f	per	8	3
Ranunculus bulbosus	Rnncls_b	f	per	37	8
Ranunculus repens	Rnncls_r	f	per	3	3

continued over ...

...continued from previous

Species	abbreviation	Plant group	Life history	No. of quadrats	No. of sites
Rhinanthus minor	Rhnnths_	f	annual	15	2
Sanguisorba minor	Sngsrb_m	f	per	130	9
Saxifraga stellaris	Sxfrg_st	f	per	1	1
Scabious columbaria	Scbs_clm	f	per	10	2
Sedum acre	Sedum_cr	f	per	1	1
Senecio squalidus	Snc_sqld	f	annual	2	2
Sesleria caerulea	Sslr_crl	g	per	52	4
Sherardia arvensis	Shrrd_rv	f	annual	8	1
Sonchus oleraceus	Snchs_lr	f	annual	1	1
Taraxacum agg.	Trxcm_gg	f	per	20	7
Thymus polytrichus	Thyms_ply	f	per	31	5
Thymus pulegoides	Thyms_plg	f	per	15	1
Tragopogon pratensis	Trgpgn_p	f	bi	1	1
Trifolium campestre	Trflm_cm	l	annual	3	1
Trifolium pratense	Trflm_pr	l	per	11	6
Trifolium repens	Trflm_rp	l	per	17	5
Trifolium scabrum	Trflm_sc	l	annual	3	1
Trifolium striatum	Trflm_st	l	annual	2	1
Trisetum flavescens	Trstm_fl	g	per	30	8
Vaccinium myrtillus	Vccnm_my	w	per	1	1
Verbascum nigra	Vrbscm_n	f	bi	7	1
Verbascum thapsus	Vrbscm_t	f	bi	5	2
Veronica chamaedrys	Vrnc_chm	f	per	18	5
Veronica serpyllifolia	Vrnc_srp	f	per	1	1
Vicia cracca	Vic_crcc	l	per	2	1
Viola canina	Viol_cnn	f	per	1	1
Viola hirta	Viol_hrt	f	per	23	5
Viola odorata	Viol_drt	f	per	4	2
Viola riviniana	Vil_rvnn	f	per	41	6

Appendix 4 Output and plots for generalised linear models: response of species richness to interaction of soil depth metrics and other environmental variables

Generalised linear models were used to investigate the modification of the species richness response to rainfall, temperature and nitrogen deposition through changing levels of soil depth metrics. The models were run using the glm() function in R package *lme4*, version 3.6.1 (2019). Plotting was carried out using the plot_model() function in R package *sjPlot*. For all models, null degrees of freedom = 10, and residual deviance was calculated on 7 degrees of freedom. Significance levels within models are not indicated.

Soil depth metrics modelled are:

- minimum soil depth
- maximum soil depth
- mean site soil depth
- range of soil depth on site
- standard deviation of soil depth measurements on site

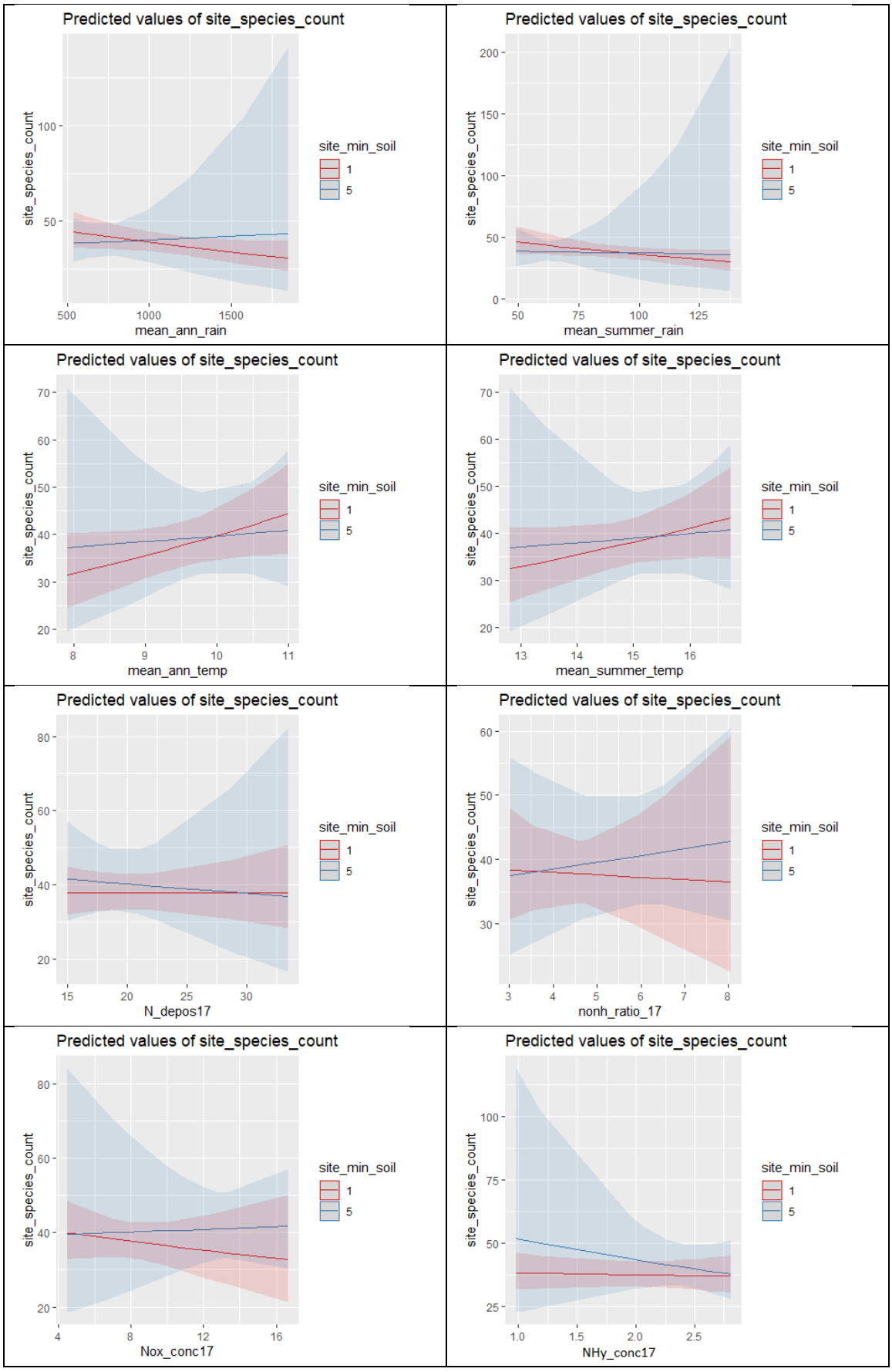
Other environmental variables within models are:

- mean annual rainfall
- mean summer rainfall
- mean annual temperature
- mean summer temperature
- mean total N deposition
- mean Nox deposition
- mean NHy deposition
- Nox/NHy ratio

Minimum soil depth

***model*: site species richness ~ minimum soil depth * mean annual rainfall**				
metric	**estimate**	**SE**	**t-value**	**p**
annual rain	-0.0004	0.0002	-1.591	0.156
minimum soil	-0.0054	0.1102	-0.775	0.464
ann rain : min soil	0.0001	0.0001	0.682	0.517
***model*: site species richness ~ minimum soil depth * mean summer rainfall**				
metric	**estimate**	**SE**	**t-value**	**p**
summer rain	-0.0057	0.0044	-1.309	0.232
minimum soil	-0.0929	0.1954	-0.475	0.649
sum rain : min soil	0.0010	0.0029	0.343	0.742
***model*: site species richness ~ minimum soil depth * mean annual temperature**				
metric	**estimate**	**SE**	**t-value**	**p**
annual temperature	0.1320	0.0901	1.465	0.186
minimum soil	0.2032	0.4097	0.496	0.635
ann temp : min soil	-0.0204	0.0411	0.496	0.635
***model*: site species richness ~ minimum soil depth * mean summer temperature**				
metric	**estimate**	**SE**	**z-value**	**p**
summer temperature	0.0861	0.0770	1.117	0.264
minimum soil	0.1875	0.5457	0.344	0.731
sum temp : min soil	-0.0122	0.0358	-0.340	0.734
***model*: site species richness ~ minimum soil depth * total N deposition**				
metric	**estimate**	**SE**	**z-value**	**p**
total N	0.0019	0.0164	0.113	0.910
minimum soil	0.0496	0.1632	0.304	0.761
total N : min soil	-0.0017	0.0082	-0.210	0.834
***model*: site species richness ~ minimum soil depth * Nox deposition**				
metric	**estimate**	**SE**	**z-value**	**p**
Nox	-0.0218	0.0306	-0.713	0.859
minimum soil	-0.0264	0.1489	-0.177	0.859
Nox : min soil	0.0052	0.0115	0.454	0.650
***model*: site species richness ~ minimum soil depth * NHy deposition**				
metric	**estimate**	**SE**	**z-value**	**p**
NHy	0.0193	0.1243	0.155	0.877
minimum soil	0.1141	0.1820	0.627	0.531
NHy : min soil	-0.0387	0.0749	-0.517	0.605
***model*: site species richness ~ minimum soil depth * Nox/NHy ratio**				
metric	**estimate**	**SE**	**z-value**	**p**
Nox/NHy ratio	-0.0191	0.0883	-0.217	0.828
minimum soil	-0.0331	0.1332	-0.249	0.803
Nox/NHy : min soil	0.0091	0.0256	0.357	0.721

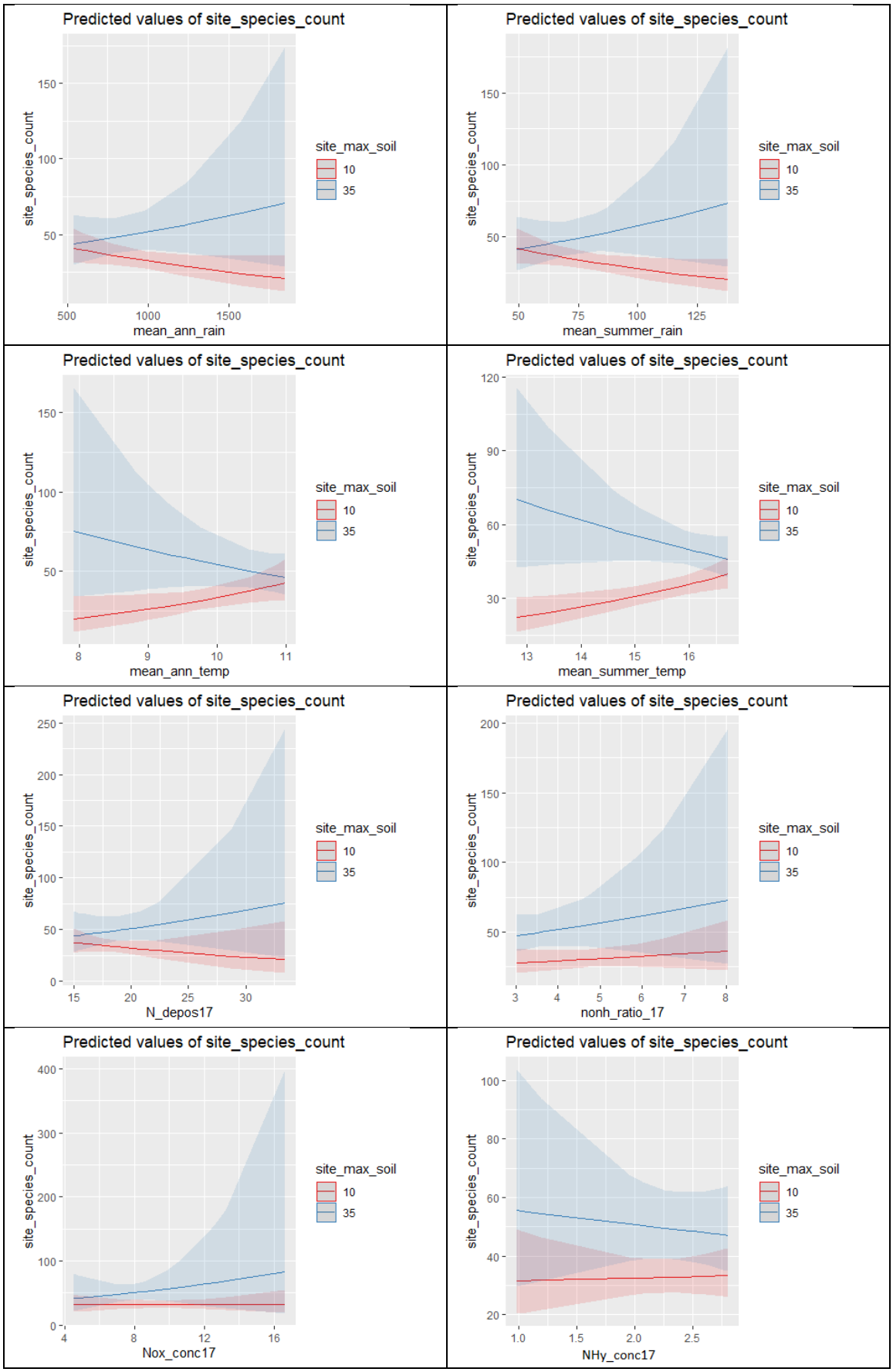
Minimum soil depth



Maximum soil depth

***model*: site species richness ~ maximum soil depth * mean annual rainfall**				
metric	**estimate**	**SE**	**t-value**	**p**
annual rain	$8.56e^{-04}$	$2.61e^{-04}$	-3.277	0.014
maximum soil	$1.64e^{-02}$	$1.21e^{-02}$	-1.352	0.219
ann rain : max soil	$3.51e^{-05}$	$1.32e^{-05}$	2.651	0.033
***model*: site species richness ~ maximum soil depth * mean summer rainfall**				
metric	**estimate**	**SE**	**t-value**	**p**
summer rain	-0.0139	0.0029	-4.728	0.002
maximum soil	-0.0289	0.0118	-2.445	0.044
sum rain : max soil	0.0006	0.0002	3.782	0.007
***model*: site species richness ~ maximum soil depth * mean annual temperature**				
metric	**estimate**	**SE**	**t-value**	**p**
annual temperature	0.4055	0.0897	4.519	0.003
maximum soil	0.1802	0.0430	4.192	0.004
ann temp : max soil	-0.0161	0.0041	-3.900	0.006
***model*: site species richness ~ maximum soil depth * mean summer temperature**				
metric	**estimate**	**SE**	**t-value**	**p**
summer temperature	0.2531	0.0988	2.256	0.038
maximum soil	0.1785	0.0772	2.313	0.054
sum temp : max soil	-0.0103	0.0048	-2.133	0.070
***model*: site species richness ~ maximum soil depth * total N deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
total N	-0.0562	0.0520	-1.082	0.315
maximum soil	-0.0302	0.0462	-0.654	0.534
total N : max soil	0.0024	0.0024	1.014	0.344
***model*: site species richness ~ maximum soil depth * Nox deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
Nox	-0.0203	0.0639	-0.319	0.760
maximum soil	0.0014	0.0320	0.043	0.967
Nox : max soil	0.0022	0.0038	0.581	0.579
***model*: site species richness ~ maximum soil depth * NHy deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
NHy	0.0780	0.2547	0.306	0.768
maximum soil	0.0273	0.0291	0.939	0.379
NHy : max soil	-0.0048	0.0121	-0.395	0.704
***model*: site species richness ~ maximum soil depth * Nox/NHy ratio**				
metric	**estimate**	**SE**	**t-value**	**p**
Nox/NHy ratio	0.0403	0.0787	0.512	0.624
maximum soil	0.0175	0.0164	1.064	0.323
Nox/NHy : max soil	0.0013	0.0040	0.325	0.754

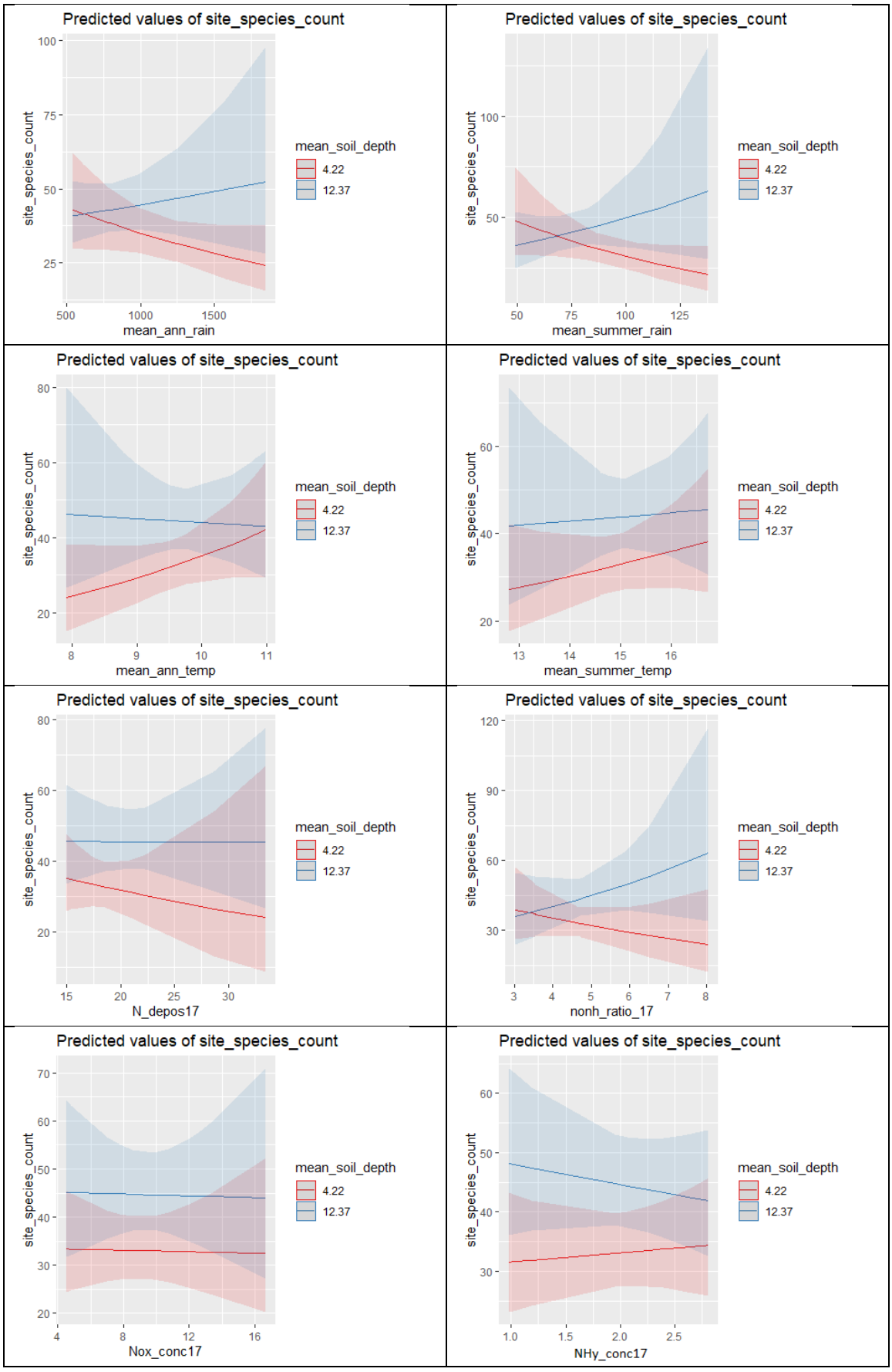
Maximum soil depth



Mean soil depth

***model*: site species richness ~ mean soil depth * mean annual rainfall**				
metric	**estimate**	**SE**	**t-value**	**p**
annual rain	$-7.47e^{-04}$	$4.07e^{-04}$	-1.859	0.105
mean soil depth	$-4.69e^{-02}$	$4.98e^{-02}$	-0.942	0.378
ann rain : mean soil	$7.64e^{-05}$	$4.87e^{-05}$	1.569	0.161
***model*: site species richness ~ mean soil depth * mean summer rainfall**				
metric	**estimate**	**SE**	**t-value**	**p**
summer rain	-0.0168	0.0066	-2.558	0.038
mean soil depth	-0.1270	0.0703	-1.808	0.114
sum rain : mean soil	0.0019	0.0008	2.235	0.061
***model*: site species richness ~ mean soil depth * mean annual temperature**				
metric	**estimate**	**SE**	**t-value**	**p**
annual temperature	0.2883	0.1644	1.754	0.123
mean soil depth	0.2796	0.1985	1.408	0.202
ann temp : mean soil	-0.0252	0.0203	-1.238	0.256
***model*: site species richness ~ mean soil depth * mean summer temperature**				
metric	**estimate**	**SE**	**t-value**	**p**
summer temperature	0.1210	0.1503	0.805	0.447
mean soil depth	0.1547	0.2873	0.539	0.607
sum temp : mean soil	-0.0080	0.0190	-0.419	0.688
***model*: site species richness ~ mean soil depth * total N deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
total N	-0.0317	0.0575	-0.551	0.599
mean soil depth	-0.0068	0.1171	-0.058	0.955
total N : mean soil	0.0026	0.0060	0.426	0.683
***model*: site species richness ~ mean soil depth * Nox deposition**				
metric	**estimate**	**SE**	**z-value**	**p**
Nox	$-2.09e^{-03}$	$5.48e^{-02}$	-0.038	0.971
mean soil depth	$3.73e^{-02}$	$6.27e^{-02}$	0.595	0.570
Nox : mean soil	$1.93e^{-05}$	$6.59e^{-03}$	-0.003	0.998
***model*: site species richness ~ mean soil depth * NHy deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
NHy	0.1100	0.2257	0.487	0.641
mean soil depth	0.0666	0.0519	1.283	0.240
NHy : mean soil	-0.0151	0.0247	-0.609	0.562
***model*: site species richness ~ mean soil depth * Nox/NHy ratio**				
metric	**estimate**	**SE**	**t-value**	**p**
Nox/NHy ratio	-0.2041	0.1734	-1.177	0.278
mean soil	-0.0865	0.1002	-0.863	0.417
Nox/NHy : mean soil	0.0255	0.0204	1.249	0.252

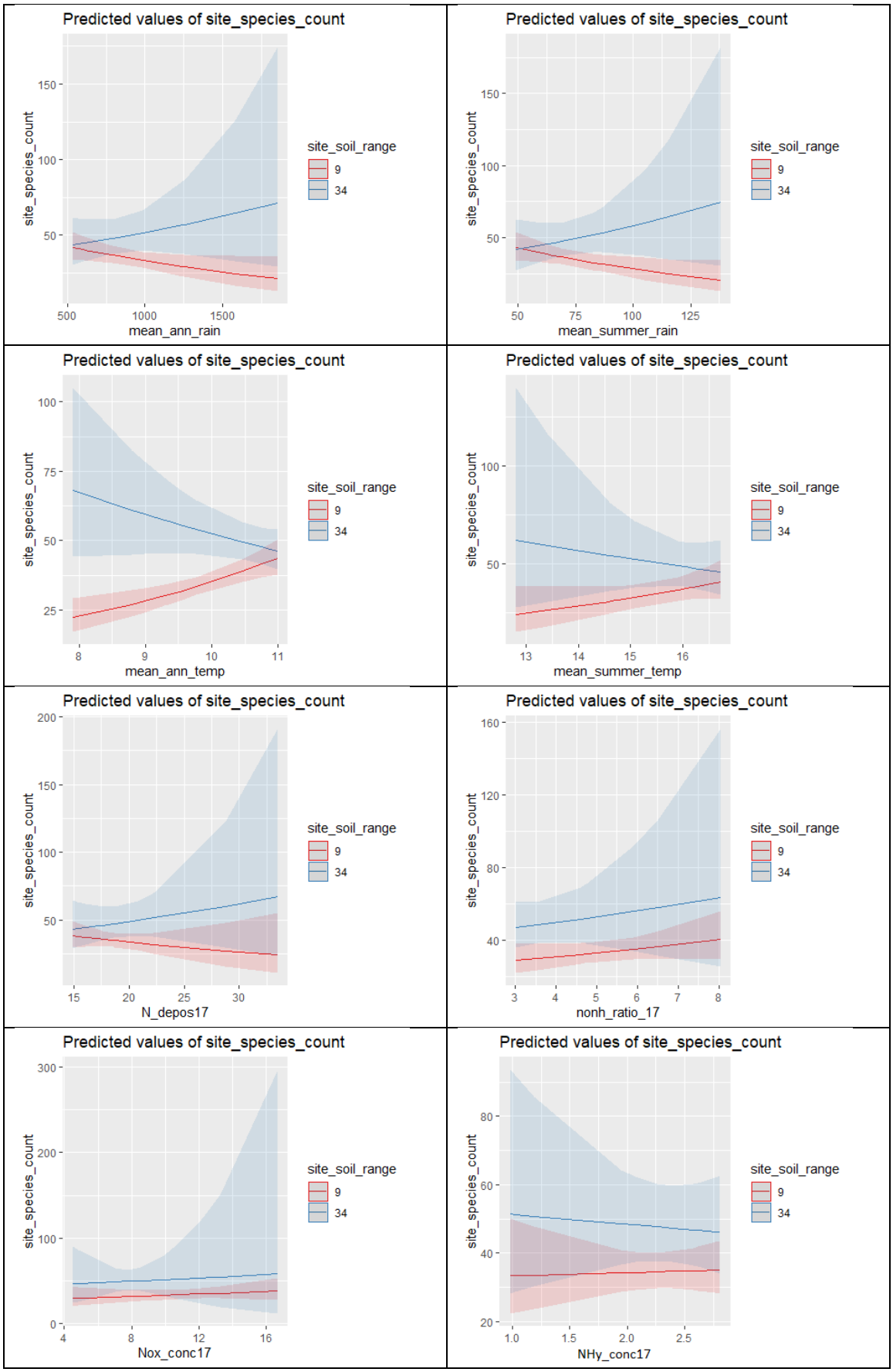
Mean soil depth



Soil depth range

***model*: site species richness ~ soil depth range * mean annual rainfall**				
metric	**estimate**	**SE**	**t-value**	**p**
annual rain	$-8.37e^{-04}$	$2.42e^{-04}$	-3.462	0.011
soil range	$-1.79e^{-02}$	$1.15e^{-02}$	-1.549	0.165
ann rain : soil range	$3.58e^{-05}$	$1.32e^{-05}$	2.706	0.030
***model*: site species richness ~ soil depth range * mean summer rainfall**				
metric	**estimate**	**SE**	**t-value**	**p**
summer rain	-0.0137	0.0025	-5.476	0.001
soil range	-0.0308	0.0105	-2.931	0.022
sum rain : soil range	0.0006	0.0001	4.233	0.004
***model*: site species richness ~ soil depth range * mean annual temperature**				
metric	**estimate**	**SE**	**t-value**	**p**
annual temperature	0.3435	0.1042	3.298	0.013
soil range	0.1538	0.0538	2.860	0.024
ann temp : soil range	-0.0138	0.0052	-2.676	0.032
***model*: site species richness ~ soil depth range *mean summer temperature**				
metric	**estimate**	**SE**	**t-value**	**p**
summer temperature	0.2081	0.1005	2.070	0.077
soil range	0.1450	0.0845	1.715	0.130
sum temp : soil range	-0.0084	0.0053	-0.583	0.158
***model*: site species richness ~ soil depth range * total N deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
total N	-0.0422	0.0458	-0.922	0.387
soil range	-0.0242	0.0439	-0.551	0.599
total N : soil range	0.0020	0.0023	0.853	0.422
***model*: site species richness ~ soil depth range * Nox deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
Nox	0.0217	0.0532	0.407	0.696
soil range	0.0187	0.0325	0.575	0.584
Nox : soil range	-0.0001	0.0038	-0.032	0.976
***model*: site species richness ~ soil depth range * NHy deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
NHy	0.0580	0.2498	0.232	0.823
soil range	0.0207	0.0303	0.682	0.517
NHy : soil range	-0.0034	0.0126	-0.274	0.792
***model*: site species richness ~ soil depth range * Nox/NHy ratio**				
metric	**estimate**	**SE**	**t-value**	**p**
Nox/NHy	0.0725	0.0660	1.100	0.308
soil range	0.0207	0.0163	1.275	0.243
Nox/NHy : soil range	-0.0004	0.0038	-0.097	0.925

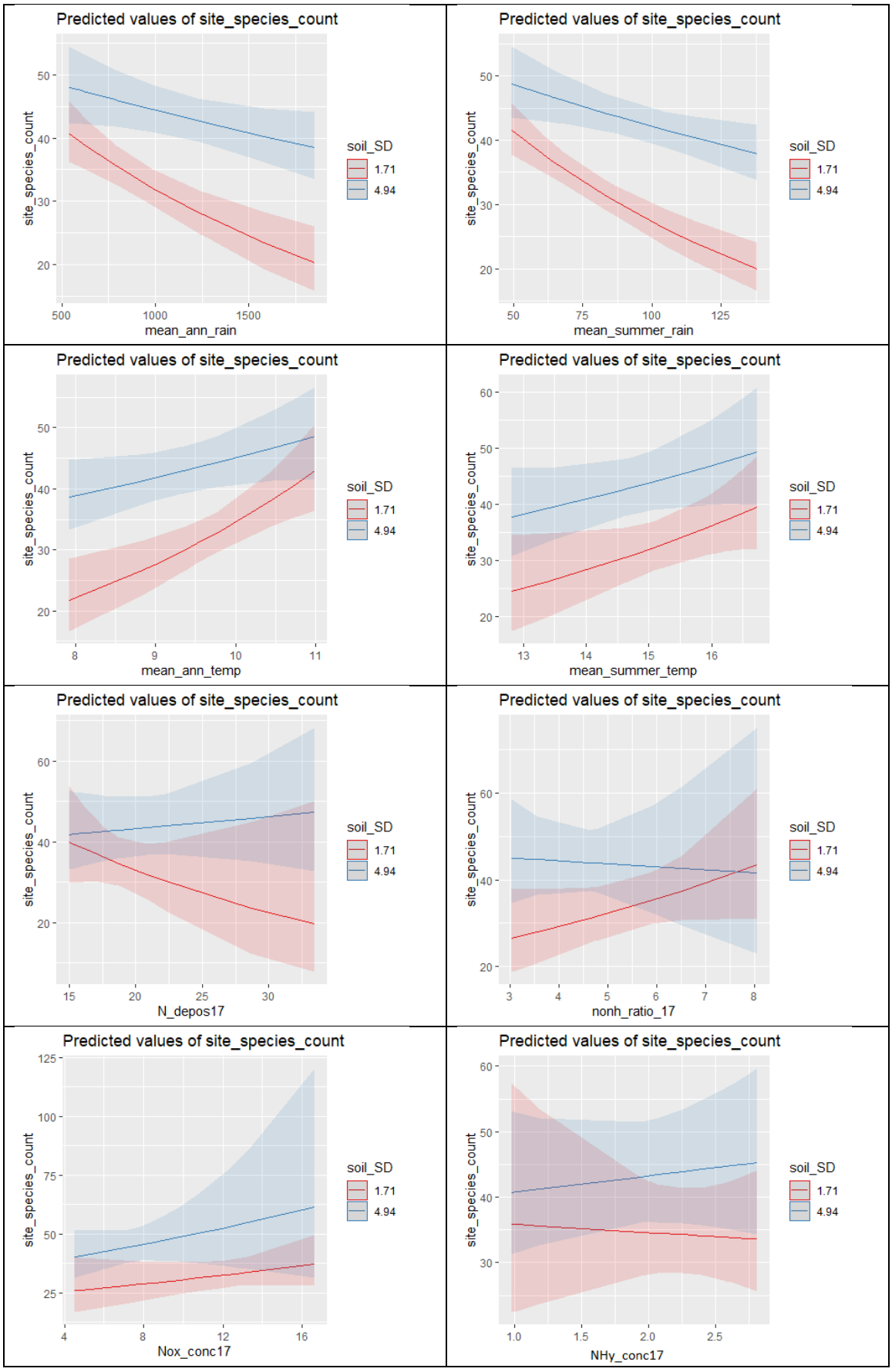
Soil depth range



Soil depth SD

***model*: site species richness ~ soil depth SD * mean annual rainfall**				
metric	**estimate**	**SE**	**t-value**	**p**
annual rain	-0.0007	0.0002	-3.574	0.009
soil SD	-0.0093	0.0549	-0.169	0.870
ann rain : soil SD	0.0001	$5.07e^{-05}$	2.219	0.062
***model*: site species richness ~ soil depth SD * mean summer rainfall**				
metric	**estimate**	**SE**	**t-value**	**p**
summer rain	-0.0111	0.0023	-4.772	0.002
soil SD	-0.0332	0.0539	-0.615	0.558
sum rain : soil SD	0.0017	0.0006	2.778	0.027
***model*: site species richness ~ soil depth SD * mean annual temperature**				
metric	**estimate**	**SE**	**t-value**	**p**
annual temperature	0.2973	0.1059	2.807	0.026
soil SD	0.5340	0.2577	2.072	0.077
ann temp : soil SD	-0.0451	0.0264	-1.707	0.132
***model*: site species richness ~ soil depth SD * mean summer temperature**				
metric	**estimate**	**SE**	**t-value**	**p**
summer temperature	0.1502	0.1061	1.416	0.200
soil SD	0.3471	0.4142	0.838	0.430
sum temp : soil SD	-0.0167	0.0274	-0.609	0.562
***model*: site species richness ~ soil depth SD * total N deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
total N	-0.0623	0.0532	-1.169	0.281
soil SD	-0.1954	0.2492	-0.784	0.459
total N : soil SD	0.0140	0.0128	1.093	0.310
***model*: site species richness ~ soil depth SD * Nox deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
Nox	0.0279	0.0484	0.577	0.582
soil SD	0.1312	0.1496	0.877	0.410
Nox : soil SD	0.0014	0.0153	0.090	0.931
***model*: site species richness ~ soil depth SD * NHy deposition**				
metric	**estimate**	**SE**	**t-value**	**p**
NHy	-0.0839	0.2923	-0.287	0.783
soil SD	0.0118	0.1634	0.072	0.945
NHy : soil SD	0.0286	0.0735	0.389	0.709
***model*: site species richness ~ soil depth SD * Nox/NHy ratio**				
metric	**estimate**	**SE**	**t-value**	**p**
Nox/NHy	0.1583	0.1205	1.313	0.230
soil SD	0.2694	0.1900	1.418	0.199
Nox/NHy : soil SD	-0.0353	0.0378	-0.934	0.382

Soil depth SD



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